Medical Research Fund

1915-16

(06)

[1915-1916]

MEMORANDUM RE MEDICAL RESEARCH FUND.

Total Receipts to 30 June, 1915,		$ 32,800.	
Received since 30 June		3,000.	$35,800.
Expended to 30 June, 1915,	$ 21,862.20		
Appropriations thus far made for 1915-16	300.00	22,162.20	
Expended on salaries of Drs. Goldie W. F. McPhedran A. Homer and of C. Greenwood to 30 Sept., 1915, (these appointments now terminated)	1,105.00		
Amount required to pay salaries of the following to 30 June, 1916,			
A. Bruce Macallum	1,200.00		
H. K. Detweiler	1,000.00		
F. S. Minns	600.00	3,905.00	
			26,067.20
Balance available 7 October, 1915,			$ 9,732.80

ADDRESS REPLY TO
PROFESSOR A. HUNTER
DEPARTMENT OF PATHOLOGICAL CHEMISTRY
UNIVERSITY OF TORONTO

UNIVERSITY OF TORONTO

November 5th, 1915.

The President,
University of Toronto.

Dear Mr. President:-

I beg to call your attention to the case of Charles Greenwood, recently laboratory assistant, under the Medical Research Fund, to Dr. Caulfeild and subsequently to Dr. Minns. Mr. Greenwood received his salary ($35.00 monthly) up to September 30th. The work for which he was engaged was not completed until October 9th, and Mr. Greenwood now desires, and in my opinion, is entitled to, payment for the period October 1 - 9 inclusive. The Bursar informs me that he is at present without authority to make this payment. I should be obliged if you would supply that authority by making the necessary recommendation to the Board of Governors.

I may say that although Mr. Greenwood's appointment was made in the first instance for one year from 1st October, 1914, there is to be found in the minutes of the Research Committee meeting for May 11th, 1915, the following recommendation:- "That C. Greenwood, the laboratory man in Dr. Caulfeild's laboratory, be retained until these experiments are completed." It was in virtue of this recommendation that Dr. Minns felt justified in retaining the services of Mr. Greenwood beyond the original period of his appointment.

I remain,

Yours sincerely,

Andrew Hunter

Professor of Pathological Chemistry.

379

University of Toronto
DEPARTMENT OF HYGIENE

TORONTO, CANADA

26th October 1915.

Dr C.K.Clarke.
Dean. Medical Faculty.
University of Toronto.
Toronto General Hospital. Toronto.

My dear Mr Dean,

In response to your request I have looked into the matter of the teaching of Bacteriology during the absence of Dr Duncan Graham, Lecturer in Bacteriology.

I would stongly advise that Dr H.K.Detweiler be given a temporary position in the Department of Pathology and Bacteriology, to be in charge of Bacteriology during the absence of Dr Graham. He might be made acting Lecturer or something of the sort. It would seem advisable that this be done at once in order the Dr Detweiler's status be definitely defined, since at present I understand he is on the Medical Research fund.

Since the classes continue very large in the first three years in Medicine, assistance should be given Dr Detweiler. It will not be possible for him to do justice to himself, the subject or the students unless he has reasonable assistance. I am sure Dr Graham would bear me out in this statement.

I shall be very glad to do anything I can to help at any time in the work of the teaching of Bacteriology in as far

University of Toronto
DEPARTMENT OF HYGIENE

TORONTO, CANADA

as my military and other duties will permit.

faithfully yours,

J. G. Fitzgerald.

Associate Professor, Hygiene.

TORONTO GENERAL HOSPITAL

SUPERINTENDENTS OFFICE

October 28th,1915.

President Falconer,
University of Toronto,
Toronto.

Dear President Falconer;-

When Dr. Fitzgerald returned from Camp I made a point of seeing him in regard to the teaching of Bacteriology,- the greater part of which is done after Christmas. The letter enclosed will explain the situation clearly.

In view of the criticism of the Research Department it would be well, I think, to have it put clearly on record that permission has been given to him to teach and Dr. Fitzgerald's suggestions regarding this matter are to the point.

Yours sincerely,

CKClarke

Dean.

ADDRESS REPLY TO
PROF. J. J. MACKENZIE
DEPARTMENT OF
PATHOLOGY AND BACTERIOLOGY
UNIVERSITY OF TORONTO

UNIVERSITY OF TORONTO

November 2nd, 1915.

Dr. A. McPhedran,
Chairman, Medical Research Committee.

Dear Doctor:-

I beg to make the following somewhat incomplete report of the research work which I have been carrying on in the absence of Dr. A.H.Caulfeild.

One hundred and four animals had been inoculated. Twenty-eight died during the period of observation. The remainder were autopsied during August and September. Photographs were taken, smears and cultures were made of the different specimens.

A complete record of this work has been made and a copy sent to Dr. Caulfeild for an analysis.

Yours very truly,

F.S. Minns

382

86 College St.,
Toronto, October 22nd, 1915.

The Committee of the Medical Research Fund,

Dear Sirs:-

In spite of the disorganization of the Staff of the Medical Clinics throughout the past year, papers including the work of the past two years have been published by Dr. J.A.Oille, Dr. F.W.Rolph, Dr. Minns, Dr. A.H.Caulfeild and Dr. William Goldie.

The work of the Tuberculosis Clinic has been hampered by the loss of three members but by reorganization and a close union that has been established with like clinics of the other hospitals and the Tuberculosis Department of the City Board of Health it is hoped that a more satisfactory control and investigation may be carried out. A new clinic has been established for the treatment of Syphilis with the idea of securing data as to the prevalence of the disease and the efficiency of treatment.

You have seen fit to appoint me for the ensuing year as Director, with a salary of $2000.00. In view of the extraordinary conditions and the difficulties in securing a staff, I beg that this money be placed at the disposal of your Committee to be expended in such ways as will further research work on occupational diseases.

Yours sincerely,

Wm Goldie

DR. FLETCHER MCPHEDRAN
151 BLOOR STREET, W.
TORONTO

—HOURS—
4-30 TO 6-00 P.M.
AND BY APPOINTMENT

October 25th, 1915.

The Secretary,
The Medical Research Committee,

Sir:-

During the last year I have been studying the reaction of the blood during certain diseases, notably in chronic nephritis with dyspnoea and also in diabetes. The cases are rather difficult to secure and so the work has been somewhat delayed but I have some material at present in the course of preparation for publication. In addition, Dr. F. W. Rolph and I hope to have a monograph ready soon on the various aspects of hyperacidity, studying the cases selected by the same electriacl method as has been used in the study of the reaction of the blood,

Yours obedient servant.

Fletcher McPhedran

Report. for the Medical Research Committee

by Annie Homer. 1914-1915.

The work undertaken during the year has been in continuation of a long series of experiments, begun in Laboratories in England, in which it was hoped to obtain evidence both as to the rôle of tryptophane in the metabolic processes of the body and as to its suspected connexion with blood pigment.

Tryptophane, an amino acid formed in the alimentary canal during the digestion of proteins, is known to be a necessary constituent of diet for the maintenance of the life of the individual; the only hint as to its fate in the animal body is furnished by the elimination of kynurenic acid (a constituent of normal dog urine) after the administration of tryptophane to dogs and rabbits.

It was difficult to see how the formation of an excretion product such as kynurenic acid could furnish a complete picture of the metabolism of an amino acid of such vital importance; the results of my experiments embodied in papers appearing in the September number of the Journal of Biological Chemistry indicate-: (1) that the young animal has a greater capacity for absorbing tryptophane than the adult, (2) that kynurenic acid cannot be regarded ~~as~~ other than as a bye product in the metabolism of tryptophane, (3) that the preliminary decomposition of tryptophane does not normally take place in the intestine and (4) that the presence of certain pathological pigments in human urines is due to disorded metabolism of tryptOphane in the intestine or elsewhere.

In order to ascertain the main line of breakdown of tryptophnae in the body, the action of various tissue enzymes on tryptophane has been tried and for this purpose it was necessary to work out a satisfactory method for the estimation of tryptophane. The results obtained indicate that the liver is the organ responsible for the decomposition of tryptophane;it is hoped to be able to isolate some product of decomposition which will throw ~~some~~ light on the problem under investigation.

I hope shortly to publish a paper on the action of tissue enzymes on tryptophane and also one on the ~~actic~~ effect of the salts of iron on the metabolism of tryptophane;the latter may be of some therapeutic interest.

TORONTO GENERAL HOSPITAL

SUPERINTENDENTS OFFICE

October 26th,1915.

Mr. F. A. Mouré,
Bursar, University of Toronto,
Toronto.

Dear Mr. Mouré;-

The President handed me a letter written by you regarding the subject of Research work at the University. I asked Dr. Hunter, who is in charge of a great portion of the research to give his statement in regard to what comes under his charge, as well as to outline in a general way the lines followed.
As you know, this research work is under the direction of a committee composed of the President, the Dean of the Faculty of Medicine, and Professors McPhedran, Macallum, Hunter, Brodie and Mackenzie. This committee outlines the policy to be followed and makes the appointments. There should be no confusion about the work as those who are interested know exactly what is being done and how much time those who are engaged are devoting to it.

You will find in Dr.Hunter's letter the names of the Fellows, duration of appointment, and salary, as well as the time spent in research.
Many of the men who formerly did this work have dropped out. For example, Dr. Fletcher McPhedran has no longer an appointment nor has Dr. Armour, as he has gone to the War. Dr. Caulfeild has also gone to the War. Dr. Simon has dropped out, Dr.Sharpe and Dr. A. Fletcher have gone to the War. Dr. Annie Homer's appointment merely runs to the end of December, when the special work she is doing will be completed.

At the present, then, the men employed are Drs. Goldie, Detweiler, Bruce Macallum, and Dr. Annie Homer. These are all engaged on work of the utmost importance.
Dr. Goldie spends half of his day in the Hospital managing the medical portion of the Out Patient Clinic, where material for research is found by the many workers in this Department.
As the result of investigations of material studied by Dr. Goldie, Dr. Oille and Dr. Detweiler, some of the most important medical discoveries of the last year or two have been made in connection with Endocarditis. This work is bringing great credit to Toronto and Dr. Detweiler, the Research Fellow who has particular charge of the Bacteriological side of the investigations, is to be highly commended. Certainly no exception can be taken to the amount of work he does in twenty-four hours, as he is one of the most indefatigable investigators I have ever met.

Dr. Caulfeild's experimental work, which is now being carried on by Dr. Minns, is of the utmost importance in the investigations regarding tuberculosis, and will, I hope, have a most important bearing on better methods of treatment than we have at present discovered.

I cannot conceive that any criticism can fairly be made of the people who have undertaken the research work in the University of Toronto. It is true that results are sometimes slow in arriving but I think that the members of the committee will agree with me that the money that has been expended has been well spent. It is not always possible for laymen to see the bearing of such investigations as those the research workers are undertaking.

Enclosed with these letters you will find reprints outlining some of the research that has been done.

Yours faithfully,

C.K. Clarke

Dean.

ADDRESS REPLY TO
PROFESSOR A. HUNTER
DEPARTMENT OF PATHOLOGICAL CHEMISTRY
UNIVERSITY OF TORONTO

UNIVERSITY OF TORONTO

386

October 23rd, 1915.

Dr. C. K. Clarke,
Dean,
Faculty of Medicine,
University of Toronto.

Dear Sir:-

In partial reply to the letter regarding the Medical Research Fund, which you showed me the other day, I beg to offer the following statement:-

(1) I am personally in charge of the work done under the Fund, so far, and so far only, as it is carried out in the Department of Pathological Chemistry.

(2) The names of the Fellows who have worked in the said Department, since the inauguration of the scheme, are shown in the appended table, in which also appears a statement of the salary paid to each, the proportion of his working time given to research, and the published results of his investigations.

(3) Accompanying this statement I submit reprints of the papers named therein.

(4) Regarding Fellows whose work has been, or is being, carried out in other Departments I can make no authoritative statement; but I give below their names and such information regarding them as I possess.

(a) Dr. Goldie was appointed in Oct. 1913 as Director of the Medical Clinic of the Out-Patient-Department at a salary of $2000. This position under the Fund he still holds.

(b) Dr. Caulfeild was appointed Fellow in Dec. 1912 at a salary of $1500, giving half of his time to research. He has published since at least 2 papers.

(c) Dr. R.G.Armour was appointed at $1500 in Jan. 1913 and gave half his time to research.

ADDRESS REPLY TO
PROFESSOR A. HUNTER
DEPARTMENT OF PATHOLOGICAL CHEMISTRY
UNIVERSITY OF TORONTO

UNIVERSITY OF TORONTO

October 23rd, 1915.

(d) Dr. Detweiler was appointed in June 1915 at $1000 and gives his whole time.

I remain,

Sincerely yours,

Andrew Hunter

Professor of Chemical Pathology.

NAME OF FELLOW.	DURATION OF APPOINTMENT.	SALARY.	TIME SPENT DAILY IN RESEARCH.
Dr. Fletcher McPhedran.	Dec.1912- Sept. 1915.	$1500	Half.
Dr. C.G.Imrie.	Oct. 1912 - Sept. 1914.	$750-1000	Whole.
Dr. N.C.Sharpe.	Oct. 1913 - Apr. 1915	$1000-1250	Whole.
Dr. K.M.B.Simon.	Oct. 1913 - Sept.1914	$750	?
Dr. Almon Fletcher.	Oct. 1914 - Apr. 1915	$750	Whole.
Dr. Annie Homer.	Oct 1914 -	$750-1500	At first half, later whole.
Dr. Bruce Macallum.	July 1915 -	$1200	Whole.

Publications of Dr. Fletcher McPhedran:

(1) (with H. Orr) "A Case of Hemolytic Jaundice with Spleno-megaly." Can.Med. Ass. Journ., 1913, p.1.

(2) "On the Hemolytic Properties of Fatty Acids, and their Relation to the Causation of Toxic Hemolysis and Pernicious Anaemia." Journ. Exp. Med., 18, p.527, 1913.

(3) "Observations on the Reaction of the Blood in Nephritic Dyspnoea." (in preparation.)

Publications of Dr. C. G. Imrie:

(1) "On Fatty Changes in the Liver, Heart and Kidney." Journ. Path. and Bacterol., 19, p.245, 1914.

(2) "On the Fat in the Blood in a Case of Lipemia." Journ. Biol. Chem., 20, p. 87, 1915.

Publications of N.C.Sharpe:

(1) (With K.M.B.Simon) "The Excretion of Nitrogen in Fever" Journ. Exp. Med., 20, p.282, 1914.

Publications of K.M.B.Simon:

(1) (With N.C.Sharpe) see above

Publications of Dr. Almon Fletcher;

(1) "Some Considerations in the Study of Infantile Tetany, with Report of a case." Arch. Int. Med.,16, p.382,1915.

(2) (with Alan Brown) "The Etiology of Tetany - Metabolic & Clinical Studies." Amer. Journ. Diseases of Children. (in the press.)

Publications of Dr. Annie Homer:

(1) "The Relation between the Administration of Tryptophane to Dogs and the Elimination of Kynurenic Acid in their Urine. Journ. Biol. Chem., 22, p.391, 1915.

(2) "Spectroscopic Examination of the Color Reactions of Certain Indol Derivatives and of the Urine of Dogs after their Administration. Journ. Biol. Chem., 22, p.345, 1915.

(3) "A Method for the Estimation of the Tryptophane Content of Proteins." Journ. Biol. Chem., 22, p.369, 1915.

Publications of Dr. Bruce Macallum:

(1) "Studies in Growth. II. On the Chemical Nature of the Growth Substances. Journ. Biol. Chem., (in the press.)

REPORT OF RESEARCH WORK BEING CARRIED ON IN BACTERIOLOGY.

Attached to this report is a copy of a paper published this summer, covering the researches carried on during the year of 1914-1915. At the present time our field covers the work of confirmation of the results reported and a further exhaustive study of the data obtained up to the present. The organisms obtained in the cases cited in our previous report are being studied both as to their biological and chemical reaction as well as their reaction upon the living body. With regard to the last, we are carrying on an extensive experimental study with a view to establishing the connection between the streptococcus viridans and allied forms and lesions of the heart valves and muscles. Rabbits are being used in this work. So far we have obtained some evidence that the organisms mentioned have especial affinity for these parts. The difficulty of producing Endocarditis in rabbits is proverbial among investigators and our efforts are not always crowned with success.

One other line which we intend giving some time to is the development, or discovery, of a chemical compound which will be highly bactericidal for these organisms. We have arranged with a synthetic chemist to have cooperative studies made in this regard.

Associated with me in all this work is Dr. W.L. Robinson, Fellow in Pathology of the University of Toronto. Furthermore, in all that pertains to the clinical side of the study in the selection of cases and in the obtaining of material from these cases for our study, we have the collaboration of Dr. William Goldie and Dr. John A. Oille. Their work is especially valuable in connection with the appended paper on Streptococcus Bacteriaemia.

H.K. Detweiler

390

Some Considerations in the Study of Infantile Tetany, with Report of a Case

ALMON FLETCHER, M.B.
Research Fellow in Clinical Medicine, University of Toronto.

Reprinted from the Archives of Internal Medicine
September, 1915, Vol. xvi, pp. 382-388

CHICAGO
American Medical Association
Five Hundred and Thirty-Five North Dearborn Street
1915

SOME CONSIDERATIONS IN THE STUDY OF INFANTILE TETANY, WITH REPORT OF A CASE *

ALMON FLETCHER, M.B.
Research Fellow in Clinical Medicine, University of Toronto.

For years it has been known that the effect of an inorganic salt on the irritability of nerve and muscle tissue is influenced by the presence of another salt in the solution. Ringer[1] was the first to discover that the twitchings of a frog's muscle produced by immersing it in an isotonic solution of pure sodium chlorid, were abolished by the addition of a small amount of calcium. J. Loeb[2] pointed out that the toxicity of a sodium chlorid solution was not due solely to the absence of calcium, but also to the presence of the sodium ion, since distilled water had no such toxic action. He also suggested that irritability depends on various ions, especially the metallic ions, Na, Ca, K and Mg, existing in definite proportion in the tissue.

But since the effect of an alteration in these proportions varies widely in different species of animals, no general law embracing the action of these ions has yet been formulated, and for particular tissues and species, the result of any salt disturbance must be determined.

In spite of this, clinical investigation in tetany has been largely directed towards a study of calcium metabolism, and the discussion of its pathogenesis has largely centered around the calcium content of the tissues. The clinical results, however, have not been satisfactory or in any way conclusive. The almost constant co-existence of rickets with infantile tetany has presented further difficulty in the interpretation of the results, and changes in the calcium balance may be secondary to the amount of calcium deposited in the bones and not due to any change in the content of the nerve substance or body tissues in general. In experimental tetany produced by parathyroidectomy, an increased loss of calcium occurs, and administration of calcium reduces, temporarily although never permanently, both the electrical irritability and the severity of the convulsions. Other salts, however, such as magnesium and strontium also produce this effect. Mac-

---

* From the Department of Pathological Chemistry, University of Toronto, and the Sick Children's Hospital, Service of Dr. Alan Brown.

[1] Ringer: Jour. Physiol., 1886, vii, 291.

[2] Loeb, J.: Am. Jour. Physiol., 1910, iii, 383; Jour. Biol. Chem., 1905, i, 427.

Callum, Lambert, and Vogel[3] were able to produce extreme nerve hyperexcitability by perfusion of an organ with blood the calcium of which had been removed by dialysis.

Clinical results have been somewhat less constant. As a general rule, during the onset of the eclampsia, a decrease in the calcium retention occurs, and the reverse sets in during convalescence. The symptoms of the disease also can often be partially checked by the injection of a calcium salt. But this loss of calcium is not always found (Stoeltzner[4]) and administering calcium either with the food or by injection is frequently without result (Haskins and Gerstenberger[5]).

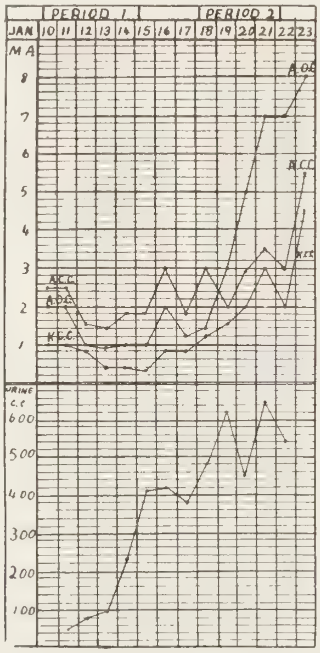


Chart showing variations in the electrical irritability and in the volume of the urine during periods of observation.

More recently, attention has been given to the effect of the other kations. It has long been the experience of pediatricians that cow's milk and particularly whey will aggravate infantile tetany; yet the organic constituents of milk (casein, fat, or sugar) have by themselves no such untoward effect. On the other hand, the inorganic salts of milk do affect the irritability, and this can frequently be demonstrated clinically. Calcium and magnesium, when administered subcutaneously, bring about, as a rule, a temporary fall in the irritability and a quiescence of the tetany. This is especially the case with magnesium, which, following the recommendations of Behrend,[6] is frequently used with considerable success as a means of combating the convulsions. The salts of the alkalies act in the opposite direction. Grulee,[7] on adding sodium or potassium chlorid to the food of spasmophilic children, obtained an increase in the irritability. Lust[8] confirmed these observations and obtained most striking results with potassium. He also records a case of tetany in which each exacerbation was accompanied by a temporary increase of weight, due to a retention of salts and fluid, the so-called metabolism edema.

---

[3] MacCallum, Lambert and Vogel: Jour. Exper. Med., 1914, xx, 149.
[4] Stoeltzner: Jahrb. f. Kinderh., 1906, lxii, 661.
[5] Haskins and Gerstenberger: Jour. Exper. Med., 1911, xiii, 314.

These facts suggest that the effect of inorganic salts on nervous tissue is not so much determined by the concentration of any one ion, but rather by their relationship to each other, and that it is some disturbance of the usual equilibrium that upsets the normal reaction of the nerve cell. A few years ago, Aschenheim[9] made estimations of the brain tissues in spasmophilia and found that, while the calcium and magnesium were most frequently decreased, sometimes they were unchanged and the sodium and potassium increased, the only constant factor being an increase in the quotient $\frac{Na + K}{Ca + Mg}$. He therefore concludes that the hyperexcitability is due to an increase in this factor. Further support of this hypothesis has been furnished by the researches of Reiss.[10]

Clinically very little work has been done to determine under what conditions these salt disturbances in tetany are induced and what the underlying factors are in their production. In recording the following case, a preliminary report of an investigation being carried on in Toronto along this line is presented.

CASE REPORT

History.—G. McK., male, aged 13½ months, was admitted Jan. 7, 1915, to the Infants' Department at the Sick Children's Hospital. Breast fed for nine months, supplemented with Allenbury's food for part of time, and then with arrowroot biscuit. Diarrhea at 3 months of age, lasting one month. Lately fed on Horlick's malted milk. Dec. 15, 1914, the first attack of tetany occurred, lasting about half a minute; described as a stiffening of the body accompanied by the spasm of the hand and foot characteristic of tetany. Before this, he had been constipated for some time and, until time of admission, bowel movements were produced only by enemata, the stools being small and hard. December 24 a more severe and prolonged attack took place, during which the child became cyanosed and seemed to struggle for breath. Similar convulsions then occurred at frequent intervals. During this period he became cross and irritable and cried almost constantly.

---

[6] Behrend: Monatschr. f. Kinderh., 1913, xii, 269.

[7] Grulee: Jour. Am. Med. Assn., 1912, lix, 938.

[8] Lust: München. med. Wchnschr., 1913, lx, 1482.

[9] Aschenheim: Monatschr. f. Kinderh., 1910, ix, 366.

[10] Reiss: Ztschr. f. Kinderh., 1911, iii, 1.

Examination.—Examination on admission showed a child under weight for age, with a fair amount of subcutaneous tissue of diminished turgor. Feet and hands in typical position of carpopedal spasm. Spleen slightly enlarged. Slight craniotabes and enlargement of the epiphyses of long bones (evidence of rickets). Cried, while being examined, continuously, with occasional crowing inspiration. Chvostek's and Trousseau's symptoms present. Muscle jerks easily elicited. Odor of acetone in breath.

Treatment.—He was offered a milk mixture made up of evaporated milk, ounces 10, barley flour, ounces 1½; lactose, ounce 1, water, up to ounces 40. Eight ounces were given every four hours, five times a day. A good deal was at first refused. Cod liver oil (20 min.) and phosphorus (gr. 1/300) three times a day. Jan. 10, 1915, he was put in a metabolism bed and observed up to January 15. During this period of five days he was obstinately constipated, having three dark, yellow, foul-smelling stools produced in each case by half an ounce of castor oil. He became more restless and irritable. The laryngospasm increased. The electrical irritability on January 10 was: K. C. C. 1, A. O. C. 2, A. C. C. 2.5, and on January 15: K. C. C. 0.4, A. O. C. 1.0, A. C. C. 1.8. The weight increased from 13 pounds 9 ounces to 14 pounds 3 ounces, and, during this period, very little urine was passed, although the amount increased towards the end of the period. Signs unchanged, no convulsions.

January 16, 1 gm. of urea was added to the food daily to stimulate the diuresis which had already begun. From January 18 to January 22 observations were again made. During this period a striking change occurred. The child took more food and became less restless; the bowels moved without the use of castor oil, and the stools became lighter in color and less offensive. The urine flow was much freer; the electrical irritability decreased from K. C. C. 1.2, A. C. C. 3, A. O. C. 1.4 on January 18 to K. C. C. 2, A. C. C. 3, A. O. C. 7 on January 22. An increase of four ounces in weight occurred The carpopedal spasm disappeared. Trousseau's sign could not be obtained, and a slight twitch only could be seen in eliciting Chvostek's symptom.

Subsequently the child continued to improve and went on to good recovery.

During the periods of observation the patient was placed in a metabolism bed, the amount of food taken each day measured, and a proportionate part used for analysis. The urine and feces were collected separately. The specimens were dried and ashed by Bunge's method. Sodium and potassium were weighed as chlorids, and separated by platinic chlorid. The calcium and magnesium were weighed as calcium oxid and magnesium pyrophosphate, following McCrudden's[11] method. The electrical irritability was determined by placing the anode or kathode over the head of the fibula and the neutral pole over the abdomen. The minimum current which would produce a contraction of the peronei was estimated in milliamperes.

The case presented an opportunity for observation during a period of intensification of the symptoms and during a period of improvement. Variations in the electrical irritability and in the urine secretion are shown in the accompanying chart.

---

[11] McCrudden: Jour. Biol. Chem., 1910, vii, 83.

The daily average estimations made of Period 1 are shown in Table 1.

TABLE 1.—DAILY AVERAGE METABOLISM ESTIMATIONS IN PERIOD 1

	Amount gm. or c.c.	Sodium Oxid	Potassium Oxid	Calcium Oxid	Magnesium Oxid
Milk..........	.....	0.1754	0.5460	0.4686	0.0324
Feces.........	14.6	0.0194	0.0387	0.0779	0.0076
Urine.........	176.0	0.0129	0.0609	0.0017	0.0216
Total loss.....	.....	0.0323	0.0996	0.0796	0.0292
Balance.......	.....	+0.1431	+0.4464	+0.3890	+0.0032
Balance in per cent. of intake	.....	81.6	81.7	83.1	9.8

A marked retention of all the salts has occurred, which would go hand in hand with the retention of fluid and the gain in weight of this period. But this retention has not been uniform, and its outstanding irregularities are, first, the large amount of potassium (0.4464 K_2O); second, the small amount of magnesium (0.0032 MgO).

The relative proportion of alkali to alkali earth $\frac{Na + K}{Ca + Mg}$ is 1.501.

The results from Period 2 were as shown in Table 2.

TABLE 2.—DAILY AVERAGE METABOLISM ESTIMATIONS IN PERIOD 2

	Amount gm. or c.c.	Sodium Oxid	Potassium Oxid	Calcium Oxid	Magnesium Oxid
Milk..........	.....	0.2706	0.8331	0.6948	0.1136
Feces.........	51.7	0.0099	0.0770	0.2478	0.0279
Urine.........	585.0	0.1157	0.5359	0.0081	0.0149
Total loss....	.....	0.1256	0.6129	0.2559	0.0428
Balance.......	.....	+0.1450	+0.2202	+0.4389	+0.0706
Balance in per cent. of intake	.....	53.2	26.4	63.2	62.1

Here the proportions are reversed. The retained potassium has been cut in half, a daily balance of 0.4464 being reduced to 0.2202, and, while the former represents 81 per cent. of the intake, the latter is only 26.4 per cent. of the potassium in the food. The sodium retention has increased 0.002 gm., but the balance in terms of the intake has decreased from 81.6 per cent. to 53.2 per cent. The calcium balance has decreased from 83.1 per cent. to 63.2 per cent. of the intake, and its absolute value has increased 0.050 gm. only; but the balance is now nearly twice that of any of the other metals. The magnesium balance has increased almost twenty-fold, i. e., from 0.0032 to 0.0706, or, in per cent. of the intake, 9.8 per cent. to 62.1. In the second period the quotient $\frac{Na + K}{Ca + Mg}$ is reduced to 0.717.

Examination of the calcium estimations by themselves would not lead one to believe that calcium changes could be held responsible for the development of tetany, and the figures demonstrate that actual loss of calcium is by no means essential for its production. In fact, the irritability has increased over a period of five days during which 0.3896 gm. of CaO were being daily retained in the tissues. Examined in connection with the other metals, the relative increase in the calcium retention during the second period becomes quite evident, being greater than the weight of the three other metals combined.

During this period of improvement, the body has been excreting the alkalies of the food and it is of special clinical interest to observe that nearly all of this excretion was performed by the kidney. This excretion occurred simultaneously with an increased flow of urine, whereas in the first period, during which the alkalies were retained, the kidneys were remarkably inactive. At the same time the restoration of the normal salt equilibrium took place along with an improvement in the gastro-intestinal system. The stools became less offensive and the bowels less constipated; the appetite improved and more food was taken. The association of fluid retention and gastro-intestinal disturbance, observed in the first period, is seen not infrequently in children, and the former seems to depend directly on the digestive disturbance since observation has shown that it can be controlled by dietetic changes, and disappears as the digestion improves. Carbohydrates and the salts of sodium and potassium are especially liable to induce these fluid changes and may well be reduced when there is any reason to believe that fluids are being retained. Taking away part of the whey from the food, as is sometimes done in the treatment of tetany, is a means of lowering the sodium and potassium intake; the calcium remains in the curd.

The importance of the kidney in regulating the salt equilibrium is evident from the figures given, and, when the urine is small in amount, the kidney may be stimulated by means of an organic diuretic—such as urea—1 gm. of which was given daily with the food.

The high magnesium balance is noteworthy in view of the specific action of magnesium upon nerve tissue. Its narcotic effect led Behrend[6] to use it in checking the symptoms of tetany. He found, however, that the magnesium, when given subcutaneously, is excreted again within twenty-four hours, and that injections must be repeated daily. A similar experience has been had in rickets, in which calcium, if injected, is not retained; yet, as convalescence begins, this loss stops and the tissues regain the power of retaining calcium. So in tetany, with improvement, the body begins to re-establish the normal salt relationship, and the ability of the tissues to hold magnesium may be an evidence of a subsidence of the disease.

To sum up briefly: Clinical observation would not warrant the conclusion that calcium changes alone account for the nerve irritability in tetany, but would seem to support the hypothesis that the tetany results from a disturbance of the concentration equilibrium of the salts.

Such salt changes are probably associated with gastro-intestinal disturbances and decreased activity of the kidney, and, as improvement in the function of these two systems occurs, restoration of the normal salt equilibrium ensues. Treatment, accordingly, from these indications should aim at restoring a normal digestion and increasing the activity of the kidney.

391

A METHOD FOR THE ESTIMATION OF THE TRYPTOPHANE CONTENT OF PROTEINS, INVOLVING THE USE OF BARYTA AS A HYDROLYZING AGENT

BY

ANNIE HOMER

(From the Lister Institute, London, and the Departments of Biochemistry and Pathological Chemistry, University of Toronto)

Reprinted from
THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. XXII, No. 2, September, 1915

Reprinted from THE JOURNAL OF BIOLOGICAL CHEMISTRY, Vol. XXII, No. 2, 1915

A METHOD FOR THE ESTIMATION OF THE TRYPTOPHANE CONTENT OF PROTEINS, INVOLVING THE USE OF BARYTA AS A HYDROLYZING AGENT.

BY ANNIE HOMER.

(From the Lister Institute, London, and the Departments of Biochemistry and Pathological Chemistry, University of Toronto.)

(Received for publication, July 9, 1915.)

INTRODUCTION.

A. Historical.

At the time that the work described in this paper was started there were very few data as to the amounts of tryptophane present in proteins, and for the lack of such data their tryptophane content was usually represented as positive or negative.

Hopkins and Cole,[1] the first investigators to isolate tryptophane from a protein digest, weighed the substance in the crystalline form and found that 1.5 per cent of tryptophane could be obtained from pure casein. Osborne and Harris[2] made use of Hopkins and Cole's glyoxylic reaction to find the relative amounts of tryptophane present in various vegetable proteins. Their observations were of a qualitative rather than a quantitative nature.

Levene and Rouiller[3] suggested that for the estimation of the tryptophane content of proteins, the digestion mixtures, made 5 per cent acid, be treated with mercury sulphate reagent; the mercury sulphate precipitate containing tryptophane be decomposed in the way described by Hopkins and Cole; and the tryptophane in solution be estimated by direct titration with bromine water in the presence of amyl alcohol. The disappearance of the characteristic purple color was taken by them as an indication of the end of the reaction. They published data with regard to the estimation of tryptophane in presence of tyrosine and cystine;

---

[1] Hopkins, F. G., and Cole, S. W., *Jour. Physiol.*, 1901–02, xxvii, 418.

[2] Osborne, T. B., and Harris, I. F., *Ztschr. f. analyt. Chem.*, 1904, xliii, 376.

[3] Levene, P. A., and Rouiller, C. A., *Jour. Biol. Chem.*, 1906–07, ii, 481.

made suggestions as to the method of procedure should these two substances be present in the digest; and stated their intention of publishing data as to the tryptophane content of proteins; but nothing further has been published by them on this subject.

Fasal[4] precipitated the tryptophane in the protein digest by means of mercury sulphate reagent and after decomposition of the mercury sulphate precipitate, estimated the amount of tryptophane in the solution by means of Hopkins and Cole's glyoxylic reaction. Using the same method he also estimated the percentage of tryptophane in malignant tumors.[5] He published the following figures for the percentage of tryptophane in various proteins: Hammarsten's casein 0.65 per cent; crude lactalbumin 3.07 per cent; and edestin 0.38 per cent.

Sanders and May[6] subjected proteins to pancreatic digestion and then inoculated the digest with fecal bacteria and colorimetrically estimated the indol thus produced. Their method assumes that the tryptophane is completely converted into indol, but such is not necessarily the case; a certain proportion of skatol and other derivatives of indol will always be simultaneously formed.

Herzfeld[7] has taken the tryptic digest of proteins and has colorimetrically estimated the tryptophane present in the digest by means of *p*-dimethylaminobenzaldehyde. He compared the color produced by the interaction of this reagent and tryptophane with the color of a solution of ammoniacal copper sulphate previously expressed in terms of a solution of pure tryptophane similarly treated with *p*-dimethylaminobenzaldehyde.

The writer of this paper adopting Herzfeld's directions has investigated the color reactions of various indol derivatives with this reagent, and has ascertained that the production of a blue color is not specific to tryptophane. A similar blue color is produced by indolacetic, indolaceturic, and indolpropionic acids and by skatol: indolaldehyde and indolcarboxylic acid give a greenish blue color while indol under the same conditions gives a red color ultimately changing to purple. Under the conditions given by Herzfeld in another paper[8] the red color of indol with this reagent does not change.

Herzfeld has estimated the tryptophane content of about 20 proteins and amongst others he quotes: casein 0.51 per cent; edestin 0.58 per cent; lactalbumin 0.91 per cent; Witte's peptone 1.25 per cent.

It will be noticed that these numbers for casein are lower than those given by Fasal and by Hopkins and Cole. Herzfeld's low results may be due to the fact that he worked with small quantities of the respective proteins (0.1 gm.) and that he continued the digestion for twenty-four hours only, and further, that he made use of a color reaction not specific to tryptophane. Hopkins and Cole showed that the maximum yield of tryptophane was obtained after seven to ten days' digestion, a fact recognized and appreciated by Plimmer and Eaves[9] in their estimation of tyrosine in protein digests. The result given by Fasal for casein is much lower than that given by Hopkins and Cole who actually isolated tryptophane in the crystalline form. It is a matter of common experience to obtain a 1 per cent yield of tryptophane from commercial casein. Fasal's numbers were based on colorimetric reactions involving the use of glyoxylic acid. From the author's observations on the factors affecting the color reaction in question[10] it is conceivable that the discrepancy between the figures quoted by Fasal and by Hopkins and Cole was due to the interference of other substances of an indol nature thrown down by the precipitating reagent.

---

[4] Fasal, H., *Biochem. Ztschr.*, 1912, xliv, 392.

[5] Fasal, *ibid.*, 1913, lv, 88.

[6] Sanders, J. A., and May, C. E., *Biochem. Bull.*, 1912–13, ii, 373.

[7] Herzfeld, E., *Biochem. Ztschr.*, 1913, lvi, 259.

[8] Herzfeld, *loc. cit.*, 82.

Finally Elizabeth Kurchin,[11] using Herzfeld's method, has estimated the tryptophane content of normal and pathological kidneys.

B. Method Adopted in the Present Investigation.

It is a matter of common experience that tryptophane can not be isolated in any appreciable extent from the products formed during an acid hydrolysis of proteins and for this reason in most of the previously described methods the protein has been hydrolyzed by trypsin. As the use of trypsin is accompanied by many disadvantages it seemed worth while to ascertain whether any other hydrolyzing agent could be used with any degree of satisfaction: it was found that baryta answered the purpose.

In the method for the estimation of the tryptophane content of proteins which is advocated in this paper the protein is hydrolyzed with baryta water. After the removal of the baryta with sulphuric acid the protein digest, made 5 per cent acid with sulphuric acid, is precipitated with mercury sulphate reagent. The precipitate is decomposed and the filtrate from the mercury sulphide is freed from substances likely to absorb bromine. The tryptophane content of the final solution thus obtained is estimated by titration with solutions both of nascent and of molecular bromine previously standardized against solutions of pure recrystallized tryptophane.

The determinations of the percentage of tryptophane in casein by the method described in this paper were made in the spring and autumn of 1913; the publication of the results has been delayed as it was desired to ascertain whether indol derivatives likely to be formed in the digest would be precipitated by the mercury reagent, and if so to what extent their formation would affect the determinations. It was also desired to make various quantitative experiments on the action of acids and alkalis on tryptophane.

---

[9] Plimmer, R. H. A., and Eaves, E. C., *Biochem. Jour.*, 1913, vii, 297.

[10] Homer, A., *ibid.*, 1913, vii, 101.

[11] Kurchin, E., *Biochem. Ztschr.*, 1914, lxv, 451.

The estimation of the tryptophane content of casein by a method involving the use of baryta as the hydrolyzing agent, of mercury sulphate as the precipitant for the tryptophane liberated during the hydrolysis, and of bromine for the final estimation of the tryptophane in solution, therefore resolved itself into the following considerations:

I. The absorption of bromine by tryptophane in aqueous solution.

II. A study of the extent to which substances other than tryptophane are precipitated by mercury sulphate reagent and of the bromine capacity of the substances thus precipitated which are likely to appear in the final solution for estimation.

III. The estimation of the tryptophane content of the hydrolysis mixtures involving: a. The treatment of the alkaline digests so as finally to obtain for bromination a solution containing the tryptophane and as free as possible from other substances likely to absorb bromine. b. The estimation of the tryptophane content of the final solutions by a determination of the ratio of their absorption of bromine to that of a solution of pure recrystallized tryptophane under the same experimental conditions.

IV. The action of acids and alkalis on tryptophane.

Section I. The Estimation of the Bromine Absorption of Tryptophane in Aqueous Solution.

The following solutions were required for the estimations:

A. Solutions of tryptophane prepared by dissolving known weights of the substance in known volumes of water.

B. A saturated solution of bromine in water. This solution was standardized (every time used) against potassium iodide and a standard solution of thiosulphate.

C. Solutions of nascent bromine prepared by the interaction (in presence of acid) of aliquot parts of the following: a solution of sodium bromate containing 15.1 grams per liter and a solution of sodium bromide containing 51.5 grams per liter.

10 cc. of each of these solutions were measured into stoppered bottles and 5 cc. of concentrated hydrochloric acid were added. After standing for some time there was added an excess of a solution of potassium iodide, and the liberated iodine was titrated with a standard solution of thiosulphate. The bromine value of the mixture of bromide and bromate was thus made.

D. A standard solution of thiosulphate ($\frac{N}{10}$): a 10 per cent solution of potassium iodide, and a freshly prepared solution of starch.

Method of Procedure.—Accurately weighed amounts of tryptophane were dissolved in known volumes of distilled water and aliquot parts of the solution were measured into stoppered flasks and treated with a known excess of an aqueous solution of bromine, or with a known amount of nascent bromine liberated by the addition of 5 cc. of concentrated hydrochloric acid to the mixture of the previously measured volumes of sodium bromate, bromide, and tryptophane solutions. The flasks were tightly corked and left at room temperature for fixed periods of time. Excess of a 10 per cent solution of potassium iodide was then added, and the flasks were recorked and allowed to stand for at least fifteen minutes. The iodine, representing the excess of bromine, was estimated by titration with standard thiosulphate solution. The difference between the amount of bromine originally added to the solution and the unabsorbed bromine finally titrated represents the bromine absorbed by the substance.

In these determinations the use of a thermostat is to be recommended as it was found that the bromine absorption of solutions of pure tryptophane at room temperature during a given period of time was greater in summer than in winter seasons of the year (England); further, to ensure constant values for the absorption of bromine it is necessary to have considerable excess of bromine and to adhere rigidly to a definite time for the reaction to take place; a variation in the time of the reaction is accompanied by slight variations in the absorption of bromine by a given weight of the substance.

It has been stated by Plimmer and Eaves that under the conditions employed by them, one molecule of tryptophane absorbs about six atoms of bromine. My results (Table I) indicate a greater absorption; one molecule of tryptophane absorbs about eight atoms of bromine, although there are indications that its bromine capacity is higher still. In the initial stages there is rapid absorption of bromine but the rate of absorption soon falls off and, as would be expected, complete saturation does not take place for some time. It will also be seen that the bromine absorption with molecular bromine is slightly higher than with nascent bromine.

From these results it is obvious that the absorption of bromine by tryptophane in aqueous solution takes place in stages and that in the complete saturation of the substance with bromine the time and temperature of the reaction must be taken into account.

Section II. The Use of Mercury Sulphate Reagent as a Precipitant and the Extent to Which the Substances Other than Tryptophane Precipitated Therewith Will Absorb Bromine.

The mercury sulphate reagent[12] according to Hopkins and Cole precipitates tryptophane, cystine, tyrosine to a certain extent, polypeptides, and other incompletely hydrolyzed products. Of these, tryptophane, tyrosine, and polypeptides will absorb bromine: there is conflicting evidence as to whether cystine absorbs bromine or not. Levene and Rouiller[3] consider that it does, whereas Plimmer and Eaves,[9] in view of the work of Knoop[13] on the absorption of bromine by amino-acids in digests, do not regard cystine as a factor to be considered in the bromination of protein digests. However, cystine, although precipitated by the mercury reagent, can not interfere because it is destroyed by the alkaline hydrolysis. To make quite sure of this point 300 cc. of the solutions as finally prepared for estimation with bromine were treated with zinc and hydrochloric acid, and the reduced liquid was tested for the presence of cystine by means of the sodium nitroprusside and the ferric chloride color reactions. Negative results were obtained, thus showing that after a baryta hydrolysis

[12] 10 per cent mercury sulphate in 5 per cent sulphuric acid.

[13] Knoop, F., *Beitr. z. chem. Phys. u. Path.*, 1908, xi, 356.

TABLE I.

The Absorption of Bromine by Tryptophane in Aqueous Solution.

Weight of tryptophane in volume of solution taken.	Time of reaction.	Weight of bromine added.	Weight of bromine residual.	Bromine absorbed by	
				1 gm.	1 gm. molecular weight
				of tryptophane.	
	min.	*gm.*	*gm.*	*gm.*	*gm.*
Mean results.					
Room temperature (summer).					
May					
0.0648	30	0.4901 (nascent)	0.2770	3.29	671
June					
0.0311	30	0.4884 (nascent)	0.3859	3.31	673
August					
0.0311	30	0.4884 (nascent)	0.3848	3.33	679
October					
0.0311	30	0.4884 (nascent)	0.3858	3.30	673
May					
0.0648	30	0.4130 (molecular)	0.1903	3.43	700
June					
0.0467	30	0.1935 (molecular)	0.0391	3.30	673
July					
0.0648	30	0.4136 (molecular)	0.1909	3.41	696
October					
0.0311	30	0.1290 (molecular)	0.0261	3.38	690
Room temperature (winter).					
0.02255	30	0.2301 (nascent)	0.1619	3.02	616
0.02255	45	0.2301 "	0.1588	3.16	645
0.02255	60	0.2301 "	0.1577	3.21	654
0.02255	2 hrs.	0.2301 "	0.1572	3.23	659
0.02255	4 hrs.	0.2301 "	0.1546	3.35	683
Temperature of reaction 17°C.					
0.0624	30	0.4602 "	0.2732	3.00	608
0.04992	2 hrs.	0.2301 "	0.0711	3.19	650
0.04721	4 hrs.	0.4683 "	0.3122	3.31	675
0.04721	7 hrs.	0.4683 "	0.3085	3.40	693
0.04721	8 hrs.	0.2301 "	0.0723	3.34	681
Temperature of reaction 17°C.					
0.0500	30	0.4720 "	0.3213	3.00	608
0.0500	1 hr.	0.4720 "	0.3136	3.17	647
0.0500	2 hrs.	0.4720 "	0.3116	3.21	655
0.0500	4 hrs.	0.4720 "	0.3037	3.36	685
0.0500	6 hrs.	0.4720 "	0.2985	3.47	708
0.0500	8 hrs.	0.4720 "	0.2990	3.46	706

of proteins and subsequent precipitation with mercury sulphate reagent there is no cystine in the precipitate.

It was found that solutions of indol, skatol, indolaldehyde, indolcarboxylic, indolacetic, indolpropionic acids, tryptophane, and the various additive compounds of tryptophane[14] when dissolved in 5 per cent sulphuric acid were all precipitated by mercury sulphate reagent. Further, it was found that all these compounds would absorb bromine under the conditions employed for the estimation of tryptophane.

In the case of tryptophane it has been shown (Table I) that the bromination is affected by conditions of time and temperature. The same is probably true for the absorption of bromine by other indol derivatives, but unfortunately, owing to scarcity of material, it was impossible to do similar time experiments with regard to their absorption of bromine: their bromine absorption after half an hour's reaction was investigated, as this was the time chosen for the bromination of the solutions obtained from the protein digests.

The solubility of indol, skatol, indolaldehyde, and indolcarboxylic acid in water is so slight that it was impossible to get reliable data as to their bromine capacity under the conditions adopted for the bromination of the final solutions obtained from the protein digests (Section III). Bromination of weighed amounts of the solids themselves did not yield concordant results.

Solutions of indol and skatol in absolute alcohol were brominated: the bromine absorption of a volume of alcohol corresponding to that used in the titrations of indol and skatol was estimated and deducted from the value for the bromine absorption of the solutions: again no concordant results could be obtained.

In another series of experiments the indol and skatol were dissolved in a small volume of absolute alcohol (25 cc.) and the solution was diluted with water to 100 cc. Aliquot parts of these solutions were treated with bromine and a correction was introduced for the bromine absorption of the alcohol. It is with diffidence that the results are published: the author does not consider that much reliance can be placed in them, they merely serve to show the order of the bromine absorption of these substances.

The following results were obtained:

---

[14] Homer, *loc. cit.*, 117.

TABLE II.

The Absorption of Bromine by Aqueous-Alcoholic Solutions of Indol and Skatol.

Weight of substance in solution.	Weight of bromine added.	Weight of residual bromine.	Weight of bromine used by volume of solvent taken.	Weight of bromine absorbed by	
				1 gm.	1 gm. molecular weight
				of substance.	
Mean results.	(Nascent)				
gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Indol					
0.0364	0.4683	0.2651	0.0745	3.54	414
0.0402	0.4620	0.2965	0.0745	3.44	402
Skatol					
0.03045	0.4683	0.3128	0.0745	2.66	348

The bromine absorption of indolacetic and indolpropionic acid in aqueous solution was also measured and the results have been embodied in Table III.

TABLE III.

The Absorption of Bromine by Indolacetic and Indolpropionic Acid in Aqueous Solution.

Weight of substance in volume of solution taken.	Weight of bromine added.	Weight of residual bromine.	Bromine absorbed by	
			1 gm.	1 gm. molecular weight
			of substance.	
Mean results. Time of reaction 30 min.				
gm.				
Indolacetic acid	*gm.*	*gm.*	*gm.*	*gm.*
0.0402	0.4856	0.3483	3.40	595
0.0400	0.3793	0.2402	3.43	600
Indolpropionic acid				
0.0400	0.4856	0.3408	3.62*	674
0.0401	0.4683	0.3358	3.30	623
0.0227	0.2295	0.1583	3.13	591

---

* The high value in this set of determinations was probably due to the fact that a small amount of sodium carbonate solution had been added to dissolve the acid.

Section III. The Baryta Hydrolysis of Casein and the Estimation of the Tryptophane Liberated Therein.

a. The Preparation from the Hydrolysis Mixture of the Solution for Estimation with Bromine.

100 grams of casein were treated with 350 grams of barium hydrate in 2.5 liters of water, and the mixture was heated on a water bath for periods varying from twenty to one hundred and twenty hours. The baryta was removed by slight excess of sulphuric acid and the liquid filtered. The filtrate was made 5 per cent acid with sulphuric acid, treated with an excess of mercury sulphate reagent and allowed to stand for forty-eight hours. The mercury sulphate precipitate may contain tyrosine, cystine, various incompletely digested protein substances, and other indol derivatives as well as tryptophane.

The tryptophane was separated from the other substances present in the mercury sulphate precipitate and likely to absorb bromine by the following process: The precipitate, after having been freed from tyrosine by repeated washing with 5 per cent sulphuric acid until the washings no longer gave Millon's test, was suspended in 2 per cent sulphuric acid and decomposed with sulphuretted hydrogen. The filtrate from the mercury sulphide was freed from sulphuretted hydrogen and while still acid (approximately 2 per cent) was treated with a solution of phosphotungstic acid with avoidance of excess. After standing, the precipitate of polypeptides, etc., if any, was filtered off; the excess of phosphotungstic acid was removed by means of baryta, and the latter by means of sulphuric acid. The liquid was again tested with Millon's reagent, and if there were any indications of tyrosine the precipitation with the mercury sulphate reagent was repeated. In the experiments under consideration it was found unnecessary to reprecipitate.

Cystine, although precipitable by the mercury reagent, can not interfere because it is destroyed by the alkaline hydrolysis.

In view of these observations (Section II) with regard to the precipitation of indol derivatives by the reagent it was necessary to make tests for the presence of indol derivatives other than tryptophane in the solutions for bromination.

The following four color tests are in vogue for the detection of indol derivatives in solution:

1. *The glyoxylic reaction* originally employed as a specific test for tryptophane, is given by all indol derivatives although the actual shade of color produced varies considerably.[10]

2. *The purple color reaction with bromine water* is specific to tryptophane and, under certain conditions, for its additive compounds. The other indol derivatives absorb bromine but do not give any characteristic color reaction with this reagent.

3. *Sodium nitrite and hydrochloric acid* give color reactions with all indol derivatives. Indol, skatol, indolaldehyde, indolcarboxylic acid, indolacetic, indolaceturic acids treated with these reagents give color reactions which are characterized by well defined absorption bands, whereas indolpropionic acid gives no characteristic color under the same conditions.[15]

4. Indol derivatives treated with Ehrlich's *p-dimethylaminobenzaldehyde* according to the directions given by Herzfeld yield characteristic color reactions. Indol gives a characteristic red color, and tryptophane, as shown by Herzfeld, gives a beautiful blue color. However in the present research it was ascertained that a similar blue color is given by indolacetic, indolaceturic, and indolpropionic acids and by skatol: indolaldehyde and indolcarboxylic acid give a greenish blue color.

The original protein digests and also the solutions finally obtained after the decomposition of the mercury sulphate precipitate were therefore tested for the presence of indol, skatol, indolaldehyde, indolcarboxylic and indolacetic acids, and for tryptophane by the color tests given above. Indolpropionic acid can not be detected in presence of tryptophane except by extraction of the solution with ether, in which tryptophane is insoluble, removal of the solvent, and conversion of the residue, if any, into the nitroso-derivative.

If indol, skatol, etc., are present in a digest, presumably they must have been formed from tryptophane during the process of hydrolysis, and they will be precipitated by the mercury reagent together with tryptophane. Owing to the slight solubility of indol, skatol, indolaldehyde, and indolcarboxylic acid there can be, at the most, but traces of these substances in the final solutions for estimation with bromine: as regards indolacetic and indolpropionic acids their absorption of bromine is so close to that of tryptophane (page 377) that for the small amounts present they will not appreciably affect the estimation: that is to say, they will influence the bromine absorption to the same extent as though they had been present as the parent substance, tryptophane.

---

[15] Homer, *loc. cit.*

As a matter of experience in fourteen hydrolyses with baryta there were three only of the final solutions which gave any indication of the presence of indol derivatives other than tryptophane. Of these, two indicated traces of indol, and one traces of indolacetic acid. Moreover, that there could not have been any appreciable conversion of tryptophane into derivatives not appearing in the final solutions is shown by the results obtained from the hydrolyses of the same sample of casein for varying periods of time (page 381) and from the results of the hydrolyses of pure tryptophane.

b. Estimation of the Tryptophane Content of the Solutions Obtained after the Decomposition of the Mercury Sulphate Precipitates, etc.

An aliquot part (50 cc.) of the solution for estimation was treated with excess of nascent or molecular bromine of known strength and the unabsorbed bromine estimated by means of potassium iodide and standard thiosulphate solution. The bromination was carried out in well stoppered bottles fitted with ground glass stoppers or with tightly fitting waxed corks. The bromine absorption of the solutions was compared with that of a freshly prepared solution of pure recrystallized tryptophane treated with the brominating solutions under comparable conditions of time and temperature; from these observations the tryptophane content of the hydrolysis mixtures could be calculated.

It was found that concordant results could be obtained if the conditions as indicated above (page 373) were kept constant during each series of experiments.

Samples of commercial casein obtained from three different firms were hydrolyzed with baryta and the hydrolysis mixture was treated in the manner described above to ascertain (α) the tryptophane content of the protein by the method under investigation and to see how it compared with the values given by other observers; (β) the length of time of hydrolysis for a maximum yield of tryptophane; and (γ) to what extent further hydrolysis would lead to a diminution of tryptophane in the final solution.

The results of the determinations have been embodied in Table IV and in each case the mean results of a series of observations have been given.

TABLE IV.

The Tryptophane Content of Commercial Casein as Estimated by the Method Described in This Paper.

Sample.	Weight of casein taken.	Time of hydrolysis.	Volume of final solution.	Bromine absorbed by final solution.		Bromine absorbed by 1 gm. tryptophane under the same conditions.	Tryptophane content of 100 gm. casein.
				(a) Nascent.	(b) Molecular.		
	gm.	*hrs.*	*cc.*	*gm.*	*gm.*	*gm.*	*gm.*
A	200	40	500	10.45		3.30	1.59
B (1)	100	20	1000	3.24		3.29	0.99
					3.64	3.44	1.06
(2)	100	40	1000	3.41		3.29	1.04
					3.85	3.44	1.1
(3)	100	60	1000	3.41		3.29	1.04
					3.75	3.44	1.09
(4)	100	80	1000	3.41		3.29	1.04
					3.88	3.44	1.13
C (1)	100	120	1000	4.07		3.30	1.24
(2)	100	120	1000	4.07		3.30	1.24

An examination of the results given in Table IV shows that except for Sample A the percentages of tryptophane indicated are all lower than those given by Hopkins and Cole but higher than those given by Fasal and by Herzfeld. Hopkins and Cole obtained 1.5 grams of tryptophane from 100 grams of Hammarsten's pure casein; but to the best of my knowledge their average yield from commercial casein was not much more than 1 per cent.

From the determinations made with Sample B it appears that the length of time suitable for a complete separation of tryptophane from casein is about forty hours.

That there could not have been any appreciable loss of tryptophane due to the formation of indol derivatives or other compounds not present in the final solution is evidenced first by the results obtained from the hydrolysis of the same sample (Sample B) for varying periods of time and secondly, by the fact that duplicate hydrolyses of Sample C conducted for the same periods of time and treated separately have given concordant results.

The solutions for estimation with bromine were all tested for the presence of indol derivatives (possibly precipitated by the reagent): where detected they were present as traces only.

The advantages in favor of the use of the above described method for the estimation of the tryptophane content of proteins are:

1. The ease with which the hydrolysis can be conducted.
2. The fact that the tryptophane does not tend to disappear during the hydrolysis.
3. The values obtained for casein are nearer those obtained by Hopkins and Cole, who actually isolated and weighed the tryptophane, than those given by investigators using colorimetric methods. In any case the values obtained in this research, in view of the possible formation of decomposition products from tryptophane and their subsequent loss in the processes employed, are doubtless too low rather than too high.
4. The method is proving of the utmost service in a series of experiments in which the action of tissue enzymes on tryptophane is being studied.

From the consideration of the question of the possible destruction of tryptophane by prolongation of the time of digestion of the protein with baryta I was led to a consideration of the action of alkalis and acids on pure tryptophane.

Section IV. The Action of Acids and Alkalis on Pure Tryptophane.

a. The Action of Acids on Tryptophane.

It is a matter of common experience that tryptophane can not be estimated in any appreciable quantity from the products formed during an acid hydrolysis of tryptophane.

The following experiments demonstrate this clearly:

1. 100 grams of casein were heated with 150 cc. of a 25 per cent solution of sulphuric acid for periods of 1, 2, 4, 8, 12, and 20 hours. Baryta was used to remove the acid and after filtration of the barium sulphate the filtrate was made 5 per cent acid with sulphuric acid and treated with mercury sulphate reagent. After washing the precipitate free from tyrosine it was found that in each case there was little more than a trace of precipitate left on the filters, at any rate not sufficient for estimation.

Thus at no period of the digestion was tryptophane present in any appreciable quantity.

In order to ascertain whether the poor yield of tryptophane in an acid hydrolysis is due to the direct action of acid on the substance as soon as it is liberated from the protein molecule the following experiments were made with pure tryptophane.

2. 25 grams of pure tryptophane dissolved in 150 cc. of a 25 per cent solution of sulphuric acid were heated on a sand bath for forty-two hours. The acid was removed by baryta and after filtration of the barium sulphate, removal of the last trace of baryta or of acid, the liquid was evaporated to crystallizing point and 12.5 grams of tryptophane were recovered. The recovery of so much tryptophane showed that the decomposing action of the acid had been slight.

In two other experiments 5.5 and 10 grams of tryptophane, to each of which had been added 1 gram of ferric sulphate, were heated on sand baths with 100 cc. of a 25 per cent solution of sulphuric acid for twenty-one and twenty-four hours respectively. The reacting liquids became deeply pigmented and in neither case was it possible to recover any tryptophane nor was any indol derivative isolated from the gummy residues although they both gave a marked glyoxylic color reaction.

3. Small weighed amounts of pure tryptophane were treated (a) with 25 per cent sulphuric acid alone, (b) with 25 per cent sulphuric acid in presence of a small amount of ferric sulphate, and (c) with the acid in presence of small amounts of copper sulphate. At the end of the time allowed for the reaction the liquids were made slightly alkaline with baryta, filtered, the precipitates well washed, and the filtrate after the removal of the baryta was made up to a certain bulk (Solution A).

An aliquot part of Solution A was made 5 per cent acid and after filtration from barium sulphate was precipitated with mercury sulphate reagent. The mercury sulphate precipitate was treated in the usual way (page 378) and the resulting neutral solution made up to a known volume (Solution B) aliquot parts of which were treated with nascent bromine.

The other portion of Solution A was made slightly alkaline with baryta and steam distilled. Both distillate and residue were tested colorimetrically for the presence of indol derivatives; the tests were negative.

In some cases the bromine absorption of both Solutions A and B was estimated with a view to ascertaining whether after the hydrolysis of tryptophane with acid there were present in the digest substances which absorbed bromine but which were not precipitated by the mercury sulphate reagent.

An examination of Table V shows that the bromine capacity of Solution A when estimated directly is considerably higher than can be accounted for by the tryptophane precipitated as the mercury sulphate compound and estimated in Solution B. It is thus obvious that there are formed during an acid hydrolysis of tryptophane substances which absorb bromine but which are not precipitated by the mercury sulphate reagent.

It is also evident that acid alone has a slow action on pure tryptophane whereas in the presence of an oxygen carrier there is considerable decomposition of the substance: for the same period of time the effect of a salt of iron is somewhat more marked than that of a salt of copper. The rate of decomposition, however, does not provide a simple explanation of the disappearance of tryptophane in acid digests.

Some observers consider that during an acid hydrolysis of tryptophane an internal anhydride is formed, but from the evidence adduced in this paper (Section II) it is clear that so long as the indol ring remains intact, it should be precipitated by mercury sulphate reagent and should absorb bromine.

The results recorded above demonstrate the presence of substances in the hydrolysis mixture which absorb bromine but which are not precipitated by the mercury sulphate reagent. It may be that the tryptophane newly split off from the protein molecule is very susceptible to chemical reaction, and it is con-

TABLE V.

The Action of 25 per cent Sulphuric Acid on Pure Tryptophane.

Kind of metallic salt added.	Weight of tryptophane taken.	Weight of metallic salt added	Time of hydrolysis.	Bromine absorption of Solution A measured *directly.*	Bromine absorption of the tryptophane content of Solution A (calculated from the absorption of bromine by Solution B).	Bromine absorbed by 1 gm. of tryptophane under the same conditions.	Tryptophane content of Solution A.	Loss of tryptophane during the hydrolysis.
	gm.	*gm.*	*hrs.*	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*
(a) —	2	—	20	6.38	6.58	3.30	1.99	0.5
(b) $Fe_2(SO_4)_3$	1.5	0.15	17	4.70	3.78	3.30	1.14	23
"	2	0.2	40	4.84	3.84	3.30	1.16	42
"	1	1	40		0.74	3.00	0.25	75
(c) $CuSO_4$	1	1	40		1.05	3.00	0.35	65

Solution A was the hydrolysis mixture after the removal of acid and metallic salt.

Solution B was prepared from the decomposition of the mercury sulphate precipitate thrown down from an aliquot part of Solution A and represents the tryptophane content of Solution A.

ceivable that during an acid hydrolysis of protein some product of hydrolysis acts as an oxygen carrier, or it may be that the other amino-acids serve as catalysers and induce the destruction of the tryptophane as quickly as it is split off from the complex molecule of the protein.

b. The Action of Baryta on Pure Tryptophane.

Before the completion of this research Herzfeld[8] had published the results of his observations on the action of alkalis on proteins and on tryptophane itself. He hydrolyzed small quantities of protein and of tryptophane itself with different alkaline solutions. After distilling the hydrolysis mixtures, he estimated the indol in the distillate. His results showed that there is slight production of indol from the action of water, more from the action of 0.5 per cent sodium carbonate solution, and a considerable amount from that of a 9 per cent solution of caustic soda in presence of copper sulphate. He states that the indol in the distillates from the baryta hydrolysis is even less than from the hydrolysis with 0.5 per cent sodium carbonate.

My experiments on the action of baryta on tryptophane and on tryptophane in presence of ferric salts recorded in this paper had been done before the appearance of Herzfeld's paper. Since then I have also tried the action of baryta on tryptophane in presence of copper sulphate. The results of my experiments confirm the observation of Herzfeld, although he estimated the indol production, whereas I estimated the tryptophane content of the hydrolysis mixtures. It will be seen that after forty hours' hydrolysis there has been practically no loss of tryptophane, and even in the presence of salts of iron and copper the loss was not very marked.

As in previous cases the hydrolysis mixtures after being freed from baryta and from copper or iron were made up to a known volume (Solution A). An aliquot part of Solution A was precipitated with mercury sulphate reagent and the remainder was used for colorimetric tests.

The mercury sulphate precipitate was decomposed in the usual way and the filtrate made up to a known volume, Solution B. Part of Solution B was estimated for its tryptophane content by means of solutions of nascent bromine: the remainder was used for colorimetric tests.

Of the final solutions, that obtained from the hydrolysis with baryta in presence of copper was the only one which showed any indication of indol, but even so the intensity of the color reaction revealed the presence of traces only.

In other experiments the hydrolysis mixtures were not used for precipitation with the mercury reagent but were made slightly alkaline with baryta and distilled; the distillates were extracted with xylol and tested with *p*-dimethylaminobenzaldehyde; there was a distinct indol coloration but the intensity of color given by the xylol extract of the distillate obtained from one-half the total bulk of the hydrolysis mixture was not as great as that given by 0.005 gram of pure indol dissolved in xylol and treated with the reagent in the same way. It was also noticed that if the baryta were completely removed from the hydrolysis mixture and the latter then made alkaline with sodium carbonate and distilled, the intensity of the color reaction of the xylol extract of the distillate was much greater than in the previous case. This observation is also in confirmation of Herzfeld's statements.

It is obvious that at the temperature at which the hydrolysis of tryptophane with baryta was conducted, there was little production of indol. Colorimetric tests failed to reveal the presence of other indol derivatives in the hydrolysis mixtures or in the final solutions for estimation with bromine.

TABLE VI.

The Action of Baryta on Tryptophane.

Kind of metallic salt added.	Weight of tryptophane taken.	Weight of metallic salt added.	Time of hydrolysis.	Bromine absorption of Solution A calculated from that of Solution B.	Bromine absorption of 1 gm. tryptophane under the same conditions.	Tryptophane content of hydrolysi mixture.	Loss of tryptophane during hydrolysis.
	gm.	*gm.*	*hrs.*	*gm.*	*gm.*	*gm.*	*per cent*
—	1	—	20	3.48	3.30	1.06	—
—	0.5	—	40	1.50	3.00	0.50	—
$Fe_2(SO_4)_3$	1	1	40	2.53	3.00	0.84	16
$CuSO_4$	0.5	1	40	1.42	3.00	0.41	18

Solution A was the hydrolysis mixture after the removal of baryta and metallic salt.

Solution B was prepared from the decomposition of the mercury sulphate precipitate thrown down from an aliquot part of Solution A and represents the tryptophane content of Solution A.

From the results indicated in Table VI it is obvious that there is practically no loss of tryptophane during the hydrolysis with baryta even for forty hours. If the hydrolysis was conducted in the presence of a relatively large amount of metallic salts there was less than 20 per cent loss during the forty hours' hydrolysis.

SUMMARY.

The baryta hydrolysis of proteins, the precipitation of the tryptophane from the hydrolysis mixtures with mercury sulphate reagent, and the subsequent bromination of the tryptophane in solution after the decomposition of the mercury sulphate precipitate is advocated as a method for the estimation of the tryptophane content of proteins for the following reasons:

1. The ease with which the hydrolysis can be conducted.

2. The separation of the tryptophane from the other substances precipitated by the reagent is simplified by the fact that cystine is destroyed during an alkaline hydrolysis.

3. Prolongation of the time of hydrolysis does not lead to a destruction of the tryptophane split off from the protein complex.

4. The results obtained by this method for the tryptophane content of casein more nearly approximate those of Hopkins and Cole who actually isolated the tryptophane in a crystalline form than do those of other observers who have based their results on colorimetric methods.

5. The precipitation of tryptophane in solution and the estimation of the tryptophane content of the filtrate from the decomposition of the mercury compound by means of its bromine absorption are proving of great service and reliability in experiments now under progress in which the action of various tissue enzymes on tryptophane is being measured.

A criticism of the method, one shared by the methods of Fasal and Herzfeld and others, can be made, *viz.*, that the reaction used in the final determinations is not specific to tryptophane but is shared by other members of the indol group. In this case the bromine absorption of other indol derivatives present in the solutions, presumably formed from tryptophane, would not affect the final results to any appreciable extent. However, as a matter of experience, it has been found that the final solutions do not contain more than traces of indol derivatives and, further, that the results obtained indicate that there is no appreciable formation of substances from tryptophane which do not appear in the final solutions for estimation with bromine.

From these considerations we see that, although the value obtained for the tryptophane content of a protein may be somewhat lower than the true value, yet it can be taken as the lowest value assignable to the protein under consideration. Such values, though only approximate, may be of the utmost service in experiments on nutrition and in the preparation of synthetic diets.

Finally a separate series of experiments has shown the greater resistance of tryptophane to the action of baryta than to the action of acids with or without the presence of oxygen carriers.

THE WAVERLY PRESS
BALTIMORE, U. S. A.

392

A SPECTROSCOPIC EXAMINATION OF THE COLOR REACTIONS OF CERTAIN INDOL DERIVATIVES AND OF THE URINE OF DOGS AFTER THEIR ADMINISTRATION

BY
ANNIE HOMER

(From the Lister Institute, London, and the Department of Biochemistry and Pathological Chemistry, University of Toronto)

Reprinted from
THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. XXII, No. 2, September, 1915

Reprinted from THE JOURNAL OF BIOLOGICAL CHEMISTRY, Vol. XXII, No. 2, 1915

A SPECTROSCOPIC EXAMINATION OF THE COLOR REACTIONS OF CERTAIN INDOL DERIVATIVES AND OF THE URINE OF DOGS AFTER THEIR ADMINISTRATION.

By ANNIE HOMER.

(From the Lister Institute, London, and the Departments of Biochemistry and Pathological Chemistry, University of Toronto.)

(Received for publication, July 7, 1915.)

A study of the literature of the subject of urinary pigments shows that the presence of a variety of chromogens has been detected in normal and pathological urines amongst which may be mentioned: urochrom, urobilin, urohematoporphyrin, urohematin, urocyanin, indirubin, indigotin, urorosein, uroerythrin, purpurin, skatol red, etc.

There is some difference of opinion as to whether the above mentioned substances represent separate pigments, or whether some of the color reactions were due to the presence of the same chromogen in an impure condition, or even to a mixture of two or more commonly occurring chromogens. It will be seen that there has been much discussion as to whether the indigotin of animals and of plants were one and the same substance; whether the indirubin of Rosin is not identical with the uroerythrin of Simon, with the urorubihematin of Harley, with the purpurin of Golding Bird, with the uromelanin of Thudichum, and with the urorosein of Sieber; whether the skatol red of Brieger and urorosein, uroerythrin, and purpurin are not the same substance; whether urobilin is a mixture of pigments, etc.

It is quite possible that a pigment noticed and named by one observer was in reality the same as that noted by another as a new substance under the special conditions of his experiments.

With a complex fluid such as urine in which there may be several chromogens present the question as to the possible identification of a pigment by its absorption spectrum is open to criticism unless precautions are taken to ensure the elimination of the other chromogens present in the fluid. A little experience with spectroscopic analysis shows that the position of the absorption bands of a substance in solution is often affected by the solvent and by the reaction of the solution. Moreover, if there are two or more substances in solution each of which has a characteristic absorption spectrum then the absorption bands of the one will be affected by the presence of the other in the solution and *vice versa.*

From considerations such as these it is obvious that, unless steps be taken to separate the pigment under observation from others in solution, varying results will be obtained and some already known chromogen will be described as an apparently new pigment precursor in the urine.

In many cases of the description and identification of pigments in the urine no exact measurements of the color reaction have been recorded; this in itself leads to confusion as neither the naked eye nor a spectroscope with the Fraunhoffer lines as the only scale indication can be used with any degree of accuracy. Moreover, many of the observations recorded have not been made under comparable conditions; this again leads to the production of contradictory results.

It was ascertained that there was a certain amount of fragmentary data as to the color reactions of some indol derivatives; in a few cases measurements had been made of the pigments obtained from the chromogens in the urine after the administration of certain indol substances and speculations as to the possibility of the precursors of certain pathological pigments being indol derivatives had been put forward.

As I was fortunate enough to have in my possession specimens of several indol compounds closely related to tryptophane, it was thought of interest and perhaps of service to make a systematic spectroscopic study of the color reactions of these compounds and of the urine of dogs to which these substances had been administered whether by mouth or by subcutaneous injection; such a study might demonstrate the connection between certain urinary pigments and indol derivatives.

It was also possible that a comparative study of the absorption spectra thus mapped out would furnish indirect qualitative evidence as to whether the absorption of tryptophane from the ali·

mentary canal normally involves its preliminary decomposition by intestinal bacteria, the quantitative aspect of which problem is being carried out in conjunction with Dr. Hopkins of Cambridge, England.

The substances investigated were: indol, skatol, indolaldehyde, indolcarboxylic acid, indolacetic, indolaceturic, and indolpropionic acids, isatin, tryptophane, and some of the condensation products of tryptophane with aldehydo- and keto-compounds.

The method of procedure was as follows.

1. For the Substances Themselves.—Dilute aqueous solutions or suspensions of the substances were treated with concentrated hydrochloric acid and, if necessary, with a few drops of a dilute solution of an oxidizing agent such as potassium nitrite, ferric chloride, hydrogen peroxide, or calcium hypochlorite. In most cases it was necessary to warm the reacting liquids for the production of the color reaction; in some the reaction took place at room temperature. The concentration of acid required for the reactions varied considerably; in each case the temperature of the reaction and the degree of acidity were adjusted so as to ensure the production of the full color reaction and to make the absorption bands as pronounced and as sharp as possible.

In most cases the pigments were extracted with an organic solvent and the extract was well washed with dilute acid; in others it was found advisable to examine the acid solutions directly.

It was noticed with all these indol compounds that an excess of the oxidizing agent transformed the color of the solutions to a yellowish or brown shade; also that the pigments in solution in organic or inorganic solvents required the presence of acidic ions for their colors to be evident; alkalis converted the color of the solutions from purple or red to brown.

2. For the Urines under Investigation.—The samples of the urines of dogs to which the above mentioned substances had been administered by mouth or by subcutaneous injection were subjected to the treatment used for the substances themselves. It was necessary to add acid to the extent of one-half to one-third of the volume of urine taken, and it was noticed that a greater amount of oxidizing agent was required than for the aqueous solutions of the substances themselves; this was to be expected.

The dogs were put on a diet on which they were not excreting the chromogen of indican. The normal urine of every dog used was tested for the presence of the chromogens of indican, urobilin, and other pigments.

It was found that the dogs employed excreted no indoxyl compounds and no urobilin on diets of bread and milk. Even after the administration of comparatively large doses of some of the above mentioned indol compounds there was no urobilin detected in the urine.

In the case of pathological human urines it was found necessary to remove urobilin by means of lead acetate solution before proceeding to an examination for indol pigments. If indican were present, the urine, after treatment with nitrite or other oxidizing agent, was repeatedly extracted with chloroform to remove all traces of indigo pigments before attempting to make an examination of the amyl alcohol-soluble pigments. After the complete removal of the chloroform-soluble substances the urine was extracted with amyl alcohol and the extract well washed with dilute acid before being examined spectroscopically.

a. A Spectroscopic Examination of the Color Reactions of Various Indol Derivatives.

An examination of Table I shows that of the simple indol compounds all except indolpropionic acid gave characteristic absorption spectra; none of them gave any indication of the production of indigo by the oxidation methods employed.

Indolaldehyde gave a color reaction with acid alone; the addition of an oxidizing agent did not affect the position of the absorption bands. The position of these bands measured with the instruments at my disposal agreed with those given by Ellinger (Table III).

It will be seen that indolaldehyde, skatol, and tryptophane have an absorption band in common, *viz:* λ549–536. Indolacetic acid has a corresponding band but the position is slightly shifted (λ552–539).

Indolacetic acid, indolaceturic acid, and tryptophane show signs of a band λ505–485 which is the characteristic absorption band of the indolcarboxylic acid pigment. It looks as though in the oxidation of these substances there is a tendency to produce indolcarboxylic acid or some product in common with that formed from the latter substance.

b. A Spectroscopic Examination of the Urine of Dogs after the Administration of Various Indol Derivatives.

An examination of Table II shows that the administration of indol and indolaldehyde was followed by a vivid indican reaction which could be detected for three days after doses of 0.1 gram of these substances.

After the administration of skatol the dog urine exhibited a well marked color reaction and the absorption band agreed with that given by Brieger and by Porcher and Hervieux for skatol red (Table III). On the second day after the dose, whether given by mouth or subcutaneously, the urine gave a well marked indican reaction.

The administration of indolcarboxylic acid was followed by the elimination of the substance in the unchanged state, a fact evidenced by the color reaction of the urine.[1]

Laidlaw and Ewins[2] have shown that the administration of indolacetic acid is followed by the elimination of indolaceturic acid. The accompanying comparison (Tables I and II) of the color reactions of the urine after the administration of indolacetic acid with the color reactions of indolaceturic acid itself lends support to the quantitative experiments of these observers. It was noticed, however, that if the urines were allowed to become "stale" then the color reactions of the urine were those of indolacetic and not of indolaceturic acid.

Peculiar interest is attached to the chromogen present in the urine after the administration of indolpropionic acid. The acid itself does not give any characteristic color reaction with an oxidizing agent, but administration of the substance to a dog is followed by the elimination of a chromogen which is very sensitive to the merest trace of acid.

The color reaction was given by the urine on addition of acid and being allowed to stand at room temperature in contact with air but the absorption spectrum exhibited under these conditions is not the same as that given by the urine after oxidation with nitrite or with ferric chloride and acid.

---

[1] The excreted indolcarboxylic acid was identified by qualitative and quantitative analyses (unpublished observations).

[2] Ewins, A., and Laidlaw, P. P., *Biochem. Jour.*, 1913, vii, 18.

The production of a cherry-red color from the urine suggested the presence of the chromogen of urorosein, but the spectroscopic examination revealed the presence of a substance different from the chromogen of urorosein.

From Table II it will be seen that incomplete oxidation of the chromogen under consideration gives rise to the bands λ557–537 and λ521–496 whereas after complete oxidation three bands were measured, *viz.*, λ587–561, λ546–533, and λ499–485 of which the band λ499–485 was the best marked. The wave length of the third band suggests that of urobilin (Garrod and Hopkins λ505–485) and the question arose as to whether this band was an inherent property of the pigment or whether it indicated the presence of urobilin, a factor which might well be introduced on account of the reaction of the animal to this substance.[3]

It was found that the unknown chromogen like urobilin was precipitated by lead acetate solution and by saturation of the urine with ammonium sulphate. The precipitated chromogen (Garrod and Hopkins' method for the separation of urobilin was used) was carefully tested for the presence of urobilin; the ether-chloroform solution showed a faint, ill defined band at about λ490. An alcoholic solution of the chromogen made alkaline with ammonia and treated with zinc chloride did not give a fluorescent solution. It was obvious that there could not have been more than a trace of urobilin present in the urine.

The precipitated chromogen when treated with acid and an oxidizing agent was converted into the colored substance having the three well marked bands described above.

From these observations it is evident that the band λ499–485 is an inherent property of the pigment and is not due to the presence of urobilin.

The production of this pigment is of interest as it probably represents some profound change in the molecule of indolpropionic acid during its sojourn in the animal body. The chemical aspect of this change is one of the problems which Dr. Hopkins and the writer of this paper are attempting to solve.

---

[3] The account of the chemical and physiological reaction of the animal body to this substance will be given in a later paper by Dr. Hopkins and the author.

Administration of tryptophane, whether in small or large amounts, and also of its condensation products with formaldehyde, with glyoxylic acid, and with pyruvic acid, was not followed by the elimination of any corresponding chromogen in the urine; there was no indication of the chromogens of indican or of any other indol pigment, of urobilin, or of any unchanged tryptophane in the urine.

The spectroscopic evidence is in favor of the statement that normally the metabolism of tryptophane in the dog does not require the preliminary formation of the substances known to be the products of bacterial action on tryptophane. In the feeding experiments under consideration as much as 10 grams of tryptophane, both in the solid form and in solution were given in single doses to a dog of 23½ pounds' weight. In this way the organs of the animal were overloaded with the substance and the chances were that any of the above mentioned bacterial products, if formed from tryptophane normally, would be present under these conditions in sufficiently large amounts to be detected in the urine by their particular color reactions as it had been observed that after the administration of the various indol derivatives the color reaction, even in the case of small doses, could be detected in the urine excreted during the next thirty-six hours or more.

In certain pathological conditions or by the adoption of some special diet whereby the flora of the intestine is altered, the metabolism of tryptophane is often affected so that there is production of indol compounds leading to the elimination of the chromogens of indigo and of indol pigments in the urine.

The pigments of clinical interest in the urine which seem to have a close connection with indol compounds are *urorosein* and *indican.*

The urorosein reaction is characteristic of the urines of patients suffering from a variety of diseases such as chlorosis, pulmonary diseases, osteomalacia, nephritis, typhoid, carcinoma of the esophagus, ulcer of the stomach, anacidity, perityphlitis, and diabetes. The production of the chromogen of this pigment from such a variety of diseases is probably due to disturbances in the intestinal tract, but at the same time it must not be forgotten that the detection of a chromogen by the production of a rose-red color is somewhat unreliable unless the position of the absorption band is measured in each case. It has been my own experience with several pathological urines sent to me as exhibiting the urorosein reaction that, if the color reaction were carefully carried out so as to get the maximum effect, the spectroscopic examination revealed the presence of indican and not of urorosein.

Nencki and Sieber,[4] Rosin,[5] and Garrod and Hopkins[6] have made careful measurements of the absorption band exhibited by urorosein so that its detection, provided the investigator possesses a spectroscope fitted with a scale, should be a simple matter.

Herter[7] investigating the case of a child with a peculiar intestinal flora whose urine exhibited a marked urorosein reaction isolated indolacetic acid as the precursor of the pigment. But Laidlaw and Ewins[2] have shown that absorption of indolacetic acid from the intestine was followed by the elimination of indolaceturic acid in the urine. Noticing the color of the urine they suggested that indolaceturic acid was the chromogen of the pigment but they did not make measurements of the absorption band exhibited by the pigment derived from indolaceturic acid or by urorosein urines.

The work described in this paper had been started some time previous to the publication of Laidlaw and Ewins' paper; these observers very kindly furnished me with a colorless sample of crystalline indolaceturic acid and so enabled me to continue my spectroscopic work without having to isolate this substance from the urine of dogs to which I had administered indolacetic acid for other purposes.

It will be seen from the accompanying tables that indolacetic acid treated with an acid and an oxidizing agent gave a characteristic absorption band λ552–539; indolaceturic acid under the same conditions gave a well marked band λ579–539. Of these two the mean position for the indolaceturic pigment agrees with that given by Nencki, Rosin, and Garrod and Hopkins for urorosein urines.

Laidlaw and Ewins' observations on the fate of indolacetic acid in the organism together with the spectroscopic evidence adduced in this paper make it unlikely that indolacetic acid is the chromogen of urorosein but at the same time the fact that Herter actually isolated indolacetic acid as the chromogen has to be accounted for.

---

[4] Nencki, M., and Sieber, N., *Jour. f. prakt. Chem.*, 1882, cxxxiv, 333.

[5] Rosin, H. R., *Arch. f. path. Anat. u. Physiol.*, 1891, cxxiii, 519.

[6] Garrod, A. E., and Hopkins, G., *Jour. Physiol.*, 1896, xx, 112.

[7] Herter, C. A., *Jour. Biol. Chem.*, 1908, ccxxxix, 253.

In order to throw some light on the subject I attempted to isolate the chromogen from human urines exhibiting the urorosein reaction. There were many difficulties in the way, amongst others the fact that so many of the urines sent to me did not yield the urorosein but the indican reaction and that in many cases (especially of chlorosis) the reaction was so slight that there was little hope of isolating a crystalline product: a fraction of a milligram of the pure substance gives an intense color reaction.

There was sent to me a sample of urine from a patient at Guy's Hospital, London, suffering from achlorhydria gastrica. In the first sample sent there was indication of the presence of indolaceturic acid (absorption band λ573–540). The urine, evaporated to half its bulk *in vacuo*, was treated according to the directions given by Laidlaw and Ewins as follows: The urine after saturation with ammonium sulphate was acidified, extracted with ether, and the ethereal layer washed with ammonium sulphate solution and then with water. The acid was shaken out of the ether with sodium carbonate solution which was withdrawn, acidified, and again saturated with ammonium sulphate and extracted with ether. The ether extract was washed as before. After the removal of the ether by evaporation a small amount of a gummy oil was left. However, this would not form a picrate in the manner described by Laidlaw and Ewins.

The gummy residue gave an intense glyoxylic reaction and on treatment with acid and an oxidizing agent gave an intense color λ553–533 which proved to be that of indolacetic acid and not of indolaceturic acid and also not that of the original urine.

It was obvious that during the concentration and subsequent treatment the chromogen in the urine had been hydrolyzed to indolacetic acid.

Further samples were sent from the same case. However, a spectroscopic examination after the removal of urobilin revealed the presence of indolacetic and not indolaceturic acids. The urine was extracted as before and an oily magma of crystals was obtained; these crystals were identified as hippuric acid. The oily magma was therefore dissolved in 5 per cent sulphuric acid and precipitated with mercury reagent. After the decomposition of the mercury precipitate in the usual way the filtrate from the sulphide was freed from sulphuretted hydrogen and from acid, and extracted with ether. The ethereal solution was well washed and after the evaporation of the ether there remained a small amount of an oily substance which slowly began to crystallize. The crystalline residue was too small in amount to be recrystallized for melting point determinations, but spectroscopic examination of the crystals indicated that the substance was indolacetic acid.

In another case of urine supposed to be exhibiting the urorosein reaction it was observed that the absorption band was λ533–524; this corresponds to neither indolacetic nor indolaceturic acid pigments and was probably due to the presence of a mixture of pigments, although to the eye the color was that of urorosein.

Moreover, it was found that with samples of the urine of dogs after the administration of indolacetic acid to them, if the urorosein test were made with the freshly excreted urine the reactions were those of indolaceturic acid, but if the urine were allowed to become "stale" the color reaction changed to that of indolacetic acid. Further, if crystalline indolaceturic acid were added to normal urine which was then allowed to stand for some days, the color reaction changed to that of indolacetic acid.

From the work of Laidlaw and Ewins and from the evidence adduced in this paper it is obvious that the chromogen of urorosein is indolaceturic acid; from the above observations it is possible that on standing the indolaceturic acid urines may undergo bacterial decomposition with formation of indolacetic acid and glycine and, further, that even starting with a urine giving the reaction for indolaceturic acid, attempts to isolate the latter substance when present in moderately small amounts may fail on account of its hydrolysis into indolacetic acid.

From considerations such as these it is easy to understand the isolation of indolacetic acid and not of indolaceturic acid by Herter as the chromogen of urorosein. Had he measured the position of the absorption band of the pigment derived from indolacetic acid he would have seen that it did not agree with that given by Rosin and others for the absorption band of urorosein.

It is thus evident that the urorosein reaction is due to the presence of indolaceturic acid in the urine which (from the observations of Laidlaw and Ewins) has been formed from indolacetic acid absorbed from the intestine. Indolacetic acid may therefore be regarded as the precursor of the pigment urorosein in the metabolic functions of the body but indolaceturic acid is the actual chromogen of the pigment: the failure to realize this point may lead to confusion in the examination of urines with a "rose-red" color reaction.

In connection with a study of the urorosein reaction it seemed necessary to consider the question of the identity of *skatol red* and *urorosein.*

Brieger[8] isolated skatol from the products obtained by the distillation of feces and showed that after administration of this substance to a dog the urine contained a chromogen which on oxidation gave rise to a pigment which he called *skatol red.* The work of Brieger, Mester,[9] and others has shown that the chromogen of skatol red is skatoxyl sulphate. The only evidence of the occurrence of skatoxyl compounds in human urines is that furnished by Otto[10] who has shown that in the early stages of diabetes mellitus indoxyl compounds could be isolated from the urine whereas in the later phases of the disease, when gastric symptoms occurred, skatoxyl compounds were excreted.

In view of the more recent work on the color reactions of the urine of patients with disordered digestive functions and of the capacity of substances, even when analytically pure, to adsorb chromogenic materials this work of Otto calls for repetition, especially as he identified the compound isolated by him by analysis of the nitrogen and sulphuric acid constituents only; analysis of the carbon and hydrogen content was also necessary in a case in which a decision was being made between compounds whose composition and molecular weights so nearly approximated.

But whether skatoxyl compounds are to be found in human urines or not, the fact remains that skatol after administration by mouth or by subcutaneous injection is eliminated as skatoxyl sulphate, and that this substance on treatment with an acid and an oxidizing agent is transformed into a pigment having an absorption band λ577–550 (Brieger). The position of this band is the same as that observed for urorosein. Brieger considered it different from indirubin but the same as the pigments urorubin, urorosein, uroerythrin, and purpurin, a view shared by Porcher and Hervieux[11] but not held by Rössler[12] and Maillard.[13]

---

[8] Brieger, L., *Ztschr. f. physiol. Chem.*, 1880, iv, 414.

[9] Mester, B., *ibid.*, 1888, xii, 130.

[10] Otto, J. G., *Arch. f. d. ges. Physiol.*, 1884, xxxiii, 607.

[11] Porcher, C., and Hervieux, C., *Compt. rend. Acad. d. sc.*, 1904, cxxxviii, 1725.

[12] Rössler, C., *Centralbl. f. inn. Med.*, 1901, xxii, 847.

[13] Maillard, L. C., *Ztschr. f. physiol. Chem.*, 1905, xlvi, 515.

Maillard considered that the skatol red of Brieger was not a pure pigment but was contaminated with indirubin and, further, that the skatoxyl eliminated in the urine does not account for the whole of the skatol injected; he suggests that some may have been converted into an indoxyl compound. Porcher and Hervieux were opposed to Maillard's views and considered that skatol becomes entirely converted into skatoxyl: they emphatically upheld their views as to the identity of skatol red and the above mentioned pigments.

The work of MacMunn and of Garrod has demonstrated that urorosein and uroerythrin are different substances (Table III).

From the work of Laidlaw and Ewins, of Herter, and from the evidence adduced in this research, it seems clear that the precursor of the urorosein pigment is indolaceturic acid. On the other hand it has been clearly demonstrated by Brieger, Otto, and Mester that the chromogen of skatol red is skatoxyl. But the evidence as to the occurrence of skatoxyl in human urines is by no means established whereas the urorosein reaction has been commonly noted and its connection with indolacetic and indolaceturic acids demonstrated.

It is thus obvious that under suitable conditions there can be isolated from the urine two distinct chromogens which produce the same color reaction. Some explanation of this phenomenon must be found.

It is possible that the same oxidation product may have been formed from these two chromogens, although the fact that the absorption spectrum of the indolacetic acid pigment is different from that of the indolaceturic acid pigment does not favor this hypothesis.

It is known that the character of an absorption spectrum depends on the atomic and molecular vibrations of the substance and that the actual position of the bands is influenced by the size, character, and weighting effect of the side chains of the ringed compounds: thus, the position of the band of the indolaceturic acid pigment is nearer the red end of the spectrum than that of the indolacetic acid pigment, and the latter nearer the red than the indolcarboxylic acid pigment. There is very little difference between the weighting of the molecules of indolaceturic acid and skatoxyl sulphate, and it is conceivable that the weighting of their respective oxidation products (which presumably belong to the same type of compound) is such that there is similarity of their absorption spectra both as regards character and position of absorption bands; but even so, until we know the type of reaction this color reaction involves it is with extreme diffidence that suggestions of this kind are brought forward.

At the same time it must be remembered that even though there is a certain similarity between the colors produced in the two cases absolute identity or a difference cannot be claimed until the colored substances themselves thus formed have been isolated and identified; for the present purposes such a course is not expedient.

However, an examination of the pigments formed from indolaceturic acid and from skatoxyl urines indicated that although at first the evidence seemed to point to their identity it was shown later that skatol red apparently consists of two pigments.

The two chromogens of the pigments whose identity was under consideration were not precipitated to any appreciable extent by the addition of lead acetate solution to the urine; they were also incompletely precipitated by saturation of the urine with ammonium sulphate.

The pigments were insoluble in chloroform, ether, and xylol but were readily soluble in ethyl and amyl alcohols.

The *indolaceturic pigment* is insoluble in chloroform to which has been added one-third its bulk of alcohol, but if the chromogen be dissolved in ethyl alcohol and oxidized with nitrite in the presence of the required amount of acid and then shaken with chloroform, the latter solvent takes up the pigment to a slight extent. The absorption band of the pigment in the chloroform-alcohol layer shows a shift in position (λ580–545). On washing the chloroform-alcohol extract with water the color is removed from the chloroform to the aqueous-alcohol layer. Reacidification of the liquids does not restore the color to the chloroform layer; this observation is contrary to Garrod and Hopkins' experience.[6]

On the other hand if a small amount of amyl alcohol were added to the chloroform the indolaceturic pigment was readily absorbed and could not be washed away with water. The solution showed a well marked band λ580–540.

The *skatol red* urines were treated with lead acetate solution and the excess of lead was removed. The clear filtrate was then oxidized in the usual way.

It was found that the skatol red pigment was insoluble in chloroform but on shaking the urine to which a small amount of ethyl alcohol had been added, there was a separation of the color into two component parts, the one soluble in the chloroform-ethyl alcohol layer as a bluish purple almost blue color and the other remaining in the aqueous acid layer as a rose-red color. After the removal of the chloroform-alcohol-soluble pigment the acid layer was extracted with amyl alcohol.

The bluish purple chloroform-alcohol-soluble pigment showed a diffuse band λ585–560 with sharp edges; the rose-red amyl alcohol extract showed a diffuse band λ560–530 and a band λ505–480.

Mixture of the chloroform-ethyl alcohol and the amyl alcohol extracts of the components of skatol red gave a band λ572–545 and in this case the band λ505–485 was hardly discernible.

It was found that an amyl alcohol-chloroform extract of the pigment obtained from the skatol red urines previously treated with lead acetate solution showed a slight shift of the band (λ575–545) but the character of the band was not that of the chloroform-ethyl-alcohol component (above): it was ill defined and diffuse. It is clear therefore that the latter extract contains a separate pigment and the position of the band λ585–560 can not be ascribed to the shifting effect of the chloroform on the position of the absorption band of skatol red.

It is evident therefore that the pigment known as skatol red can be resolved into two component parts; such a separation could not be accomplished with the indolaceturic pigment.

A further point of dissimilarity was the comparative stability of skatol red both in acid-aqueous and in acid-amyl-alcohol solutions whereas the characteristic color of urorosein urines and of the pigment from the isolated indolaceturic acid fades very rapidly, even from concentrated solutions in acid-amyl-alcohol.

Moreover, it was ascertained that after the administration of indolacetic acid to dogs there was no trace of indican in the urine. In the case of skatol given to a dog who was on a diet on which he was excreting no indoxyl compounds, no indican could be detected in the first day's urine but the chloroform extract was reddish purple in color and showed an absorption band λ535–560; this was probably the indirubin referred to by Maillard as contaminating Brieger's skatol red. On the second day the urine showed evidence of the formation of indoxyl compounds (positive indican reaction) and on this day the skatol red reaction was much less vivid than on the first day. Both the indican and skatol red reactions were faint on the third day.

It is possible that the bulk of the skatol is eliminated as skatoxyl sulphate while a small amount is converted into indoxyl sulphate. In the first day's urine, in the presence of excess of skatoxyl, the indoxyl combines with part of the skatoxyl to form a compound of the indirubin type while in the second day's urine, the skatoxyl compound being present in a much less amount, the indoxyl follows the usual path of indigotin formation.

From these considerations it is probable that skatol red and urorosein, although they possess similar absorption spectra are not identical. The experimental evidence shows that the skatol red of the urine is not a simple pigment but a mixture of pigments which, when present together, mutually affect each other's absorption so as to produce an absorption band corresponding to that of urorosein.

The indican reaction is exhibited by the urine of dogs excreted for several days following after the administration of indol and indolaldehyde. After the administration of skatol the indican reaction is not evidenced until the second day. In the case of indol and indolaldehyde the color reaction is very intense and persists for some time after their administration (Table II). The question as to the production of indican in the urine is one of interest in view of these observations.

The chromogen of indican is a sulpho- or gluco-compound of indoxyl and is to be found normally in the urine of herbivora and in pathological human urines. While Ellinger considers that the indoxyl compounds of the urine owe their origin solely to the bacterial production of indol from tryptophane in the intestine, Rosenfeld states that under certain conditions there appear, in the urine of rabbits, indoxyl compounds which can only have been produced from the breakdown of the tissues and not from the intestinal decomposition of tryptophane. Ellinger, criticizing Rosenfeld's experimental evidence,[14] states emphatically that the indican of the urine comes solely from indol formed in the intestine, a view also held by Salkowski, Underhill, Porcher, and others.[15]

---

[14] Blumenthal, F., and Rosenfeld, F., *Charité-Ann.*, 1903. xxvii, 46. Ellinger, A., *Ztschr. f. physiol. Chem.*, 1903, xxxix, 44. Ellinger, A., and Gentzen, M., *Beitr. z. chem. Phys. u. Path.*, 1904, iv, 171. Also, Rosenfeld, F., *ibid.*, 1904, v, 83.

[15] Underhill, F. P., *Am. Jour. Physiol.*, 1903-04, x, p. xxvii. Salkowski, E., *Ztschr. f. physiol. Chem.*, 1904, xlii, 213.

The indican reaction seemed to be a feature of many of the pathological urines sent to me. In some cases the reaction was slight; in others, especially in the urine of patients suffering from pernicious anemia and cirrhosis of the liver, the reactions were most vivid.

The experimental data which I am slowly accumulating point to the liver as being the seat of the decomposition of tryptophane. It is quite feasible that in pathological cases such as those cited above, the presence of indoxyl compounds in the urine is not of necessity due to the bacterial disturbances in the intestinal tract but may owe its origin to the formation of indolaldehyde in the liver as a result of the failure of the disordered liver cells to deal normally with tryptophane.

SUMMARY.

A study of the absorption spectra of the colored substances obtained by the action of certain reagents on indol derivatives has been made and correlated with those derived from the chromogens eliminated in the urine of dogs to which these indol derivatives had been administered. The results show that:

1. Of the various indol derivatives administered, indol, skatol, indolaldehyde, indolcarboxylic and indolacetic acids undergo little change in their passage through the animal body. Apparently with indolpropionic acid there are more deep seated changes taking place.

2. Tryptophane under normal circumstances is apparently not broken down into any of the above mentioned substances before absorption from the alimentary canal.

3. The *urorosein* reaction is due to the presence of indolaceturic acid in the urine which has been produced from indolacetic acid in the intestine, the latter probably being formed as the result of bacterial action on tryptophane. Under some conditions the urorosein reaction is changed to that of indolacetic acid.

4. The pigment known as *skatol red* has the same absorption spectrum as urorosein and some observers consider them to be identical. The evidence adduced in this research shows that they are different. Skatol red consists of a mixture of two pigments which can be isolated by the use of suitable solvents; further, the

stability and solubility of the mixed pigments are different from those of urorosein.

5. The indican reaction is given markedly by the urine of dogs within a short time after the administration of indol and indolaldehyde and persists for some days. This color reaction is not given until the second day after the administration of skatol.

No indican could be detected in the urine after the administration of other indol derivatives.

6. The presence of indoxyl compounds in pathological human urines may be due to: (a) disorders of the liver whereby the liver cells may be incapable of dealing with tryptophane beyond the stage of indolaldehyde, or (b) the absorption from the intestinal tract of indol or of indolaldehyde produced from tryptophane by the action of intestinal bacteria.

TABLE I.

Spectroscopic Examination of the Color Reactions of Indol Derivatives.

Substance.	Treatment.	Solvent for pigment.	Absorption bands.
Indol	HCl and KNO_2	Amyl alcohol	λ528.5–490 (concentrated solution). λ528.5–505 (dilute solution).
Skatol	HCl and KNO_2	Amyl alcohol	λ546.5–533.5 and a diffuse ill defined band λ494–505.
		$CHCl_3$	λ546.5–533.5 and a diffuse ill defined band λ494–505.
Indolaldehyde	HCl (concentrated)	HCl aqueous	λ549–536.5 and a strong band λ495–480.
	HCl and KNO_2	HCl aqueous	λ549–533.5 and λ494.5–480.
		HCl aqueous	(Dilute solution) λ546–527.5 and λ494.5–475 (well marked).
	HCl and $FeCl_3$	Amyl alcohol	λ549–533.5 and λ499.5-475.
Indolcarboxylic acid	HCl and KNO_2	Amyl alcohol	λ505–484.
Indolacetic acid	HCl and KNO_2	HCl aqueous	λ552–539 (faint) and λ505–490 (faint).
		Amyl alcohol	λ557–539 and λ505–492.
	HCl and $FeCl_3$	Amyl alcohol	λ552–531 and λ505–492.
	HCl and KNO_2	Amyl alcohol	λ552–531 and λ505–492.
Indolaceturic acid	HCl and KNO_2	Amyl alcohol	λ579–539 (strong) and λ502–485 (very faint).
	HCl and H_2O_2	Amyl alcohol	λ579–539 and λ502–492.
	HCl and $FeCl_3$	Amyl alcohol	λ579–539 and λ502–492.
Tryptophane	Slow oxidation with $FeCl_3$ in presence of air and acid	$CHCl_3$	λ570-471 (faint).
	HCl and KNO_2	Amyl alcohol	Ill defined bands λ552–534 and λ505–485.
	HCl and $FeCl_3$	Amyl alcohol	λ549–531 and λ502–485.

TABLE I—*Concluded*

Substance.	Treatment.	Solvent for pigment.	Absorption bands.
Condensation products of tryptophane with:			
1. formaldehyde	HCl (concentrated)	Acid aqueous	Glyoxylic color reaction.
	HCl and KNO_2	Amyl alcohol	Yellowish brown solutions.
	HCl and $FeCl_3$	Amyl alcohol	
	HCl and H_2O_2	Amyl alcohol and also $CHCl_3$	No bands.
2. glyoxylic acid	HCl and KNO_2	Amyl alcohol	Yellowish brown solutions.
	HCl and $FeCl_3$	Amyl alcohol	
	HCl and H_2O_2	Amyl alcohol and also $CHCl_3$	No bands.
3. pyruvic acid	HCl and KNO_2	Amyl alcohol	Yellowish brown solutions.
	HCl and $FeCl_3$	Amyl alcohol	
	HCl and H_2O_2	Amyl alcohol and also $CHCl_3$	No bands.
Isatin	HCl and KNO_2	Amyl alcohol	Brown solution. No bands.
	HCl and $CaOCl_2$	$CHCl_3$	No bands.

TABLE II.

*Spectroscopic Examination of the Urine of Dogs after the Administration of Various Indol Derivatives.**

Substance administered.	Treatment of urine.	Solvent for pigment.	Absorption bands.
Indol (by mouth)	HCl and $CaOCl_2$	$CHCl$	λ627–598 (indican).
(by injection)	HCl and $CaOCl_2$	$CHCl$	λ627–598 (indican).
	HCl and KNO_2	Amyl alcohol	No other pigment could be detected even three days after the dose.
Skatol† (by mouth and by injection)	HCl and $CaOCl_2$	$CHCl_3$	No indican first day.
	HCl and $FeCl_3$	$CHCl_3$	Purple color λ560–535 first day.
		$CHCl_3$	λ627–598 second day. Indican.
Skatol† (by mouth and by injection)	KNO_2 and HCl	Amyl alcohol	λ572–545. More dilute 565–546.
Component 1		*Chloroform-alcohol* solution: sharp band λ585–560; solution almost blue in color.	
Component 2		*Amyl alcohol* solution: diffuse and ill defined band λ560–530 and another λ505–480; this latter is not due to the presence of urobilin. Amyl alcohol solution almost rose-red in color.	
Chloroform-amyl alcohol solution of mixture of 1 and 2 shows band λ575–545.			
Indolaldehyde (by mouth)	HCl and $CaOCl_2$	$CHCl_3$	λ627–598 (well marked).
	HCl and $FeCl_3$	$CHCl_3$	λ627–598.
	HCl and KNO_2	Amyl alcohol after removal of pigments soluble in $CHCl_3$	None.
Indolcarboxylic acid (by mouth)	HCl and $CaOCl_2$	$CHCl_3$	None (no indican).
	HCl and $FeCl_3$	$CHCl_3$	None.
	HCl and KNO_2	Amyl alcohol	λ505–484.

TABLE II—*Continued.*

Substance administered.	Treatment of urine.	Solvent for pigment.	Absorption bands.
Indolacetic acid (by mouth and by injection)	HCl and $CaOCl_2$ HCl and $FeCl_3$	$CHCl_3$	None (no indican).
Fresh urine	HCl and KNO_2	Amyl alcohol	λ570–542 and λ505–490.
	HCl and H_2O_2	Amyl alcohol	λ570–542 and λ505–490.
	HCl and $FeCl_3$	Amyl alcohol	λ570–542 and λ505–490.
Stale urine	HCl and KNO_2	Amyl alcohol	λ550–533 and λ505–490.
	HCl and H_2O_2		
	HCl and $FeCl_3$		
Normal urine to which had been added:			
1. indolacetic acid	HCl and KNO_2	Amyl alcohol	λ555–533 and λ505–490.
2. indolaceturic acid	HCl and KNO_2	Amyl alcohol	λ570–542 and λ505–490.
3. indolaceturic acid, and allowed to stand for some time	HCl and KNO_2	Amyl alcohol	λ552–534 and λ505–490.
Indolpropionic acid (by mouth and by injection)	Acidified urine allowed to stand in contact with air	Amyl alcohol	λ521–496 (very marked) and λ557–537.
	Kynurenic acid precipitated from urine (Capaldi) colored red	Ether or ethyl alcohol extract of kynurenic acid precipitate	λ527–500.
	HCl and $CaOCl_2$ HCl and $FeCl_3$	$CHCl_3$	None except in one case when after injection on the third day there was a band λ625–598 (indican).
	HCl and KNO_2	Amyl alcohol	Three well marked bands. λ587–561, λ546–533, and λ499–485 (best marked).
	HCl and H_2O_2	Amyl alcohol	
	HCl and $FeCl_3$	Amyl alcohol	
Tryptophane (by mouth and by injection)	HCl and $CaOCl_2$	$CHCl_3$	None (no indican).
	HCl and $FeCl_3$	$CHCl_3$	None.
	HCl and KNO_2	Amyl alcohol	None; ill defined brown color.
	HCl and H_2O_2	Amyl alcohol	
	HCl and $FeCl_3$	Amyl alcohol	

TABLE II—*Concluded.*

Substance administered.	Treatment of urine.	Solvent for pigment.	Absorption bands
Formaldehyde, glyoxylic acid, and pyruvic acid condensation products with tryptophane (by mouth)[*]	HCl and $CaOCl_2$ HCl and $FeCl_3$ HCl and KNO_2	$CHCl_3$ Amyl alcohol	None. None; ill defined brown color.
Isatin (by mouth)	HCl and $CaOCl_2$ HCl and KNO_2	$CHCl_3$ Amyl alcohol	None. None; ill defined reddish brown color.

[*] Normal dog urine, with the diet chosen, gave negative tests for urobilin, indican, and other pigments with characteristic absorption spectra. Rabbit urine was taken and tested for indican and the band measured.

The absorption band of indican extended from λ624–598.

[†] The skatol pigment was fractionally extracted and was separated into two components, the one soluble in chloroform-alcohol and the other in amyl alcohol.

TABLE III a.

Absorption Bands of Pigments Derived from Indol Derivatives Previously Described by Other Observers.

Substance.	Observer	Absorption bands
Indolaldehyde (with HCl)	Ellinger	λ540–530 and λ490–480.
Indolacetic acid	Salkowski	λ550–530.

TABLE III b.

Absorption Bands of the Pigments Obtained from Reactions with Urine Previously Described by Other Observers.

Substance.	Observer.	Absorption bands.
Indican	Bouma De Negri	λ630–590. λ620–559.
Indirubin	Bouma De Negri Rosin	λ562–527. λ580–535. λ589–474 (maximum 578).
Uroerythrin	MacMunn Garrod	λ546–481. In presence of H_2SO_4 λ586–552; in presence of HCl λ608–517. In alcoholic solution λ546–520 and λ506–481.
Urohematoporphyrin	Garrod	In acid solution: λ597–587; λ576–570; λ557–541.
Urorosein	Garrod and Hopkins Rosin Nencki	λ561–540; in $CHCl_3$ λ582.5–550. λ538 (maximum). λ557 (maximum).
Skatol red	Brieger Porcher and Hervieux	λ577–550. λ577–550.

TABLE IV.

*Pigments Exhibited by a Few of the Pathological Urines Submitted for Investigation.**

Case.	Urobilin.	Indican.	Other pigments after treatment with KNO_2 and HCl.
Chlorosis 1	Yes	Yes	λ573–546 (urorosein).
2	Yes	Yes	None.
3	Yes	Very marked	Ill defined band λ524 504.
Pernicious anemia	Yes	Vivid reaction	None.
Pernicious anemia	Yes	Vivid reaction (no oxidizing reagent required)	None.
Cirrhosis of the liver	Yes	Vivid reaction	Ill defined band in the green.
Achlorhydrin gastrica 1	Yes	No	λ573–540 (urorosein).
An attempt to isolate the chromogen resulted in the separation of a substance having an absorption			λ552–533 (indolacetic pigment).
Achlorhydrin gastrica 2	Yes	No	λ552–533 (indolacetic pigment).
Achlorhydrin gastrica 2 Isolated crystalline residue			λ552–534 (indolacetic pigment).
Achlorhydrin gastrica 3	Yes	No	λ533–524 (? a mixture of pigments).

---

* If the urines gave a marked urobilin reaction they were treated with lead acetate before being tested for the presence of indol pigments. If the urines contained the chromogen of indican, after oxidation with nitrite and acid, they were repeatedly extracted with chloroform to remove this pigment before being extracted with amyl alcohol.

THE WAVERLY PRESS
BALTIMORE, U. S. A.

393

THE RELATION BETWEEN THE ADMINISTRATION OF TRYPTOPHANE TO DOGS AND THE ELIMINATION OF KYNURENIC ACID IN THEIR URINE

BY

ANNIE HOMER

(From the Physiological Laboratories, University of Cambridge, the
Physiological Laboratory, University College, London, the
Lister Institute, London, and the Departments of
Biochemistry and Pathological Chemistry,
University of Toronto)

Reprinted from
THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. XXII, No. 2, September, 1915

Reprinted from The Journal of Biological Chemistry, Vol. XXII, No. 2, 1915

THE RELATION BETWEEN THE ADMINISTRATION OF TRYPTOPHANE TO DOGS AND THE ELIMINATION OF KYNURENIC ACID IN THEIR URINE.

By ANNIE HOMER.

(From the Physiological Laboratories, University of Cambridge, the Physiological Laboratory, University College, London, the Lister Institute, London, and the Departments of Biochemistry and Pathological Chemistry, University of Toronto.)

(Received for publication, July 7, 1915.)

It is known from the work of Willcock and Hopkins[1] and from that of Osborne and Mendel[2] that tryptophane is an essential constituent of diet for the maintenance of the life of an animal and it is generally accepted that there is some close connection between tryptophane and blood pigment although up to the present this conjecture has not received the support of experimental evidence.

The only direct indication of the fate of this substance in the animal body has been furnished by Ellinger[3] who showed that in dogs and in rabbits the excretion of kynurenic acid was increased by the administration of tryptophane by the mouth or by subcutaneous injection: in the human being there was no excretion of kynurenic acid after the administration of tryptophane by the mouth.

The formation of kynurenic acid from tryptophane presents an interesting problem, as it involves the conversion of an indol into a quinoline compound. Kynurenic acid has hitherto been regarded as γ-hydroxy-β-carboxytryptophane,[4] and both Ellinger and Dakin have suggested possible ways of formation of the acid from tryptophane. Ellinger regards indol glyoxylic acid as an intermediate stage in the conversion whereas Dakin considers the formation of indolpyruvic acid as a prelude to the opening of the pyrrol ring thus:

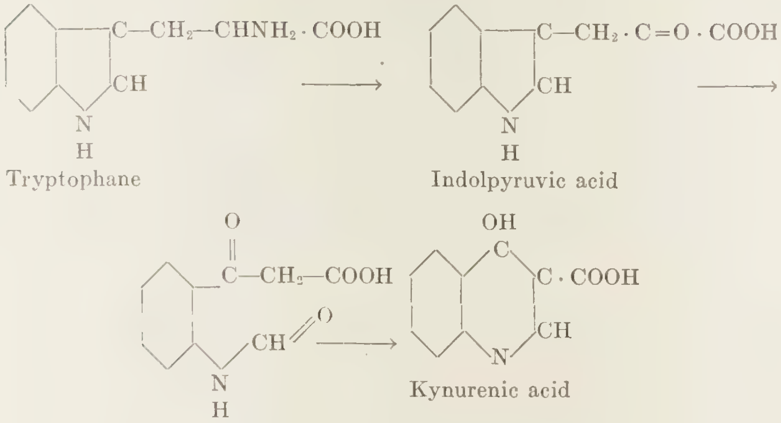
---

[1] Willcock, E. G., and Hopkins, F. G., *Jour. Physiol.*, 1906–07, xxxv, 88.

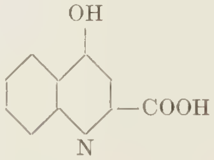
[2] Osborne, T. B., and Mendel, L. B., *Jour. Biol. Chem.*, 1914, xvii, 325.

[3] Ellinger, A., *Ztschr. f. physiol. Chem.*, 1904–05, xliii, 325.

[4] Camps, R., *ibid.*, 1901, xxxiii, 390.



But in view of the fact that kynurenic acid has been shown to be γ-hydroxy-α-carboxy quinoline[5] these suggestions are no longer tenable. However, for the present the author is not prepared to offer any suggestion as to the method of formation of this compound from tryptophane.



Before proceeding to certain metabolism experiments, undertaken conjointly with Dr. Hopkins of Cambridge, England, in which it was desired to obtain some evidence of the way in which tryptophane becomes converted into kynurenic acid in the metabolic processes of the dog, it seemed advisable to ascertain whether the kynurenic acid eliminated represented a complete picture of the metabolism of tryptophane in the body or whether a certain amount of the tryptophane administered became "side-tracked" into kynurenic acid while the remainder was utilized in furnishing either the energy or the particular compounds necessary for the maintenance of the life of the individual.

With this object in view varying doses of tryptophane were administered to dogs of different sizes and ages and the output of kynurenic acid in the urine was estimated by Capaldi's method[6] as follows:

---

[5] Homer, A., *Jour. Biol. Chem.*, 1914, xvii, 509.

> The urine was treated with half its bulk of a 10 per cent solution of barium chloride to which had been added 5 per cent concentrated ammonia. After standing for half an hour the liquid was filtered and the filtrate evaporated to half the bulk of the original volume of urine taken for the estimation. The residual liquid was decomposed with a 4 per cent solution of hydrochloric acid and allowed to stand for a period of sixteen to twenty-four hours. The crystalline precipitate was filtered, washed with dilute hydrochloric acid, dissolved in ammonia, and the excess of ammonia removed by evaporation on a water bath. The solution of kynurenic acid in ammonia was then treated with a 4 per cent solution of hydrochloric acid and allowed to stand for six hours. The crystalline precipitate was filtered through a Gooch crucible, washed, dried at 110 C., and weighed.

In one series of determinations (Table I a) in which a dog was being fed on lean meat and in which, during the preliminary period the dog was excreting traces only of kynurenic acid, it was noticed that, in the period after the removal of the tryptophane from the diet, there was apparently an appreciable amount of kynurenic acid being excreted. An examination of this supposed kynurenic acid showed that the substance was practically pure uric acid.

It was evident that the determination of kynurenic acid in the urine by Capaldi's method could not be relied upon in presence of uric acid. In order to get a true idea of the amount of kynurenic acid in the urine it was necessary therefore, either to modify Capaldi's method so as to eliminate the possible contamination of the precipitate with uric acid, or to make separate estimations of the uric acid content of the Capaldi precipitates, or to choose a diet for the dogs on which they would excrete an amount of uric acid insufficient to interfere with the estimations.

In one series of experiments (Table I b) in which a dog on a meat diet was given constant doses of tryptophane, the kynurenic acid (admixed with uric acid) was estimated by Capaldi's method (pptes. A) and the uric acid content of the precipitate was estimated as follows:

> The kynurenic-uric acid precipitates (pptes. A) were dissolved in ammonia and the excess of ammonia was evaporated off on the water bath.

---

[6] Capaldi, A., *Ztschr. f. physiol. Chem.*, 1897, xxiii, 92.

The solution was made slightly alkaline with ammonia and saturated with Kahlbaum's pure recrystallized ammonium chloride. The precipitated ammonium urate was decomposed and ultimately weighed on a Gooch crucible as uric acid.

At the same time determinations of the total amount of uric acid present in the urine were made by Hopkins' method in order to ascertain whether there was any simple relationship between the amount of uric acid present in the urine and that carried down with the Capaldi precipitates: there was none.

In view of the interference of uric acid in the estimation of kynurenic acid by Capaldi's method and the labor involved in the subsequent estimations of the uric acid content of the mixed precipitates of kynurenic and uric acids, it seemed worth while to determine whether uric acid was excreted to any appreciable extent in the urine of dogs on a diet of a lower purine value.

Of all the observers who have studied the question of the origin of kynurenic acid in the metabolic processes of the dog, amongst whom may be mentioned Liebig, Schmidt, Schmeiderberg, Schultzen, Hauser, Voit and Reiderens, and Solomin, the last mentioned alone seems to have recognized that uric acid was excreted by dogs in any appreciable amount.

Solomin[7] made determinations of the relative amounts of kynurenic acid and uric acid excreted by dogs on a diet of 400 gm. of horse flesh, 250 cc. of milk, and 250 gm. of bread a day. He determined the total output of uric acid by the Salkowski-Ludwig method and the kynurenic acid by Capaldi's method. For a dog of about 9 kg. weight the average output of uric acid was 0.11 gm. and of kynurenic acid 0.30 gm. a day.

It will be seen (Tables I a and I b) that the dogs on a diet of 500 grams of meat per day were excreting somewhat larger amounts of uric acid than were excreted by the dogs in Solomin's experiments. Further, it was found (Tables IV a, b, and c) that traces only of uric acid were excreted by dogs on a diet of bread and milk, of bread and milk and a small amount of meat (25 to 50 grams), of dog biscuits alone, and on a starvation diet.

If a dog can be induced to take any of these diets with regularity then Capaldi's method can be used for the estimation of the kynurenic acid content of the urine. The results thus obtained will give a true representation of the acid present and will not be too high on account of its contamination with uric acid.

[7] Solomin, P., *ibid.*, 1897, xxiii, 501.

Since the presence of uric acid interferes with the estimation of kynurenic acid it was necessary to ascertain whether the estimation of uric acid by (a) Hopkins' and (b) by Folin's methods could be relied on in presence of kynurenic acid.

(a) Mixtures of uric and kynurenic acids were taken. The mixture of acids was dissolved in the least possible quantity of sodium carbonate solution and the uric acid estimated by Hopkins' method.

Mixture of acids.		Uric acid precipitated as ammonium urate and estimated as uric acid by	
Weight of uric acid taken.	Weight of kynurenic acid taken.	1. Titration with $KMnO_4$ solution.	2. Weighing on a Gooch crucible.
gm.	*gm.*	*gm.*	*gm.*
0.1209	0.1232	0.1202	
0.2458	0.1506	0.2432	
0.3406	0.1256		0.3320
0.1980	0.1720		0.1920

There was no indication of the contamination of the final precipitates of uric acid with kynurenic acid.

(b) Mixtures of uric and kynurenic acids were taken and the uric acid was estimated by Folin's colorimetric method.

Three separate solutions of weighed amounts of pure uric and kynurenic acids were made by dissolving the mixed acids in a solution of lithium carbonate and making the volume up to 100 cc. Aliquot parts of these solutions (2 cc.) were treated with Folin's phosphotungstic reagent and estimated colorimetrically against a standard solution of pure uric acid treated in the same way.

Mixture of acids.		Weight of uric acid in total volume of solution estimated colorimetrically by Folin's method.
Weight of uric acid taken.	Weight of kynurenic acid taken.	
gm.	*gm.*	*gm.*
0.100	0.100	0.100
0.100	0.100	0.998
0.100	0.100	0.101

From these results it is obvious that the presence of kynurenic acid does not interfere with the estimation of uric acid by Hopkins' and by Folin's methods.

In the accompanying tables are given the results of the estimations of the amount of kynurenic acid eliminated in the urine of dogs after the administration of tryptophane. Tables I a, b, and c deal with the administration of repeated similar doses of tryptophane to dogs and puppies; Tables II a, b, and c with the administration of increasing doses of tryptophane to dogs and to puppies and a consideration of the output of total nitrogen during the tryptophane feeding; Table III, with the administration of large doses of tryptophane; Tables IV a, b, and c with the excretion of uric acids by dogs on various diets.

Before attempting to draw any conclusion as to the significance of the data given in these tables with regard to the possibility of the elimination of kynurenic acid being of the nature of a side issue, and not representing the main line of breakdown of tryptophane in the animal body, it was necessary to ascertain whether kynurenic acid, once formed, could be further dealt with by the body: in other words, does the kynurenic acid isolated from the urine represent the total amount produced from the tryptophane absorbed?

Hauser[8] administering kynurenic acid to dogs by the mouth and by subcutaneous injection was able to recover in one case, 36 per cent and in another case, 56 per cent of the tryptophane given. He found that after the administration of the substance to man none could be recovered from the urine. On the other hand Solomin[7] found that there was as much as 90 per cent of the kynurenic acid administered by injection excreted in the unchanged form by dogs and 60 per cent by rabbits. The amount of the substance recovered after administration by the mouth was much less for both animals. In the case of man none or only a very small percentage of the substance could be recovered after administration by the mouth.

It is possible that the samples of kynurenic acid used by Solomin, isolated by Capaldi's method from the urine of dogs kept on a high meat diet for some time, were contaminated with uric acid. The administration of a "mixed" product would lead to results showing a higher loss than had actually taken place. In Hauser's experiments with dogs a considerable loss was shown; here again the investigator may not have been dealing with a pure product.

---

[8] Hauser, A., *Arch. f. exper. Path. u. Pharmakol*, 1895, xxxvi, 1.

With Hauser's results indicating a possible utilization of at least 50 per cent of the kynurenic acid administered, it was necessary to ascertain whether, with pure kynurenic acid, the results would confirm those of Solomin or those of Hauser.

One of my dogs who had been on varying amounts of tryptophane was put on a diet on which he was excreting traces only of uric acid and no kynurenic acid. Doses of chemically pure kynurenic acid prepared according to the directions given in a previous paper (Homer)[5] were administered to the dog. The kynurenic acid content of the urine excreted during the thirty-six hours following the administration of the substance was estimated by Capaldi's method.

Weight of kynurenic acid administered.	Weight of kynurenic acid isolated from the urine by Capaldi's method.
gm.	*gm.*
0.690 (subcutaneously)	0.630
0.330 (by mouth)	0.266

These results support the view that the body of the dog does not utilize the kynurenic acid to any appreciable extent; whence it follows that, in the consideration of the experimental results recorded in the accompanying tables, the output of kynurenic acid can be taken as a true representation of the total amount of kynurenic acid formed by the body from the tryptophane administered to the animal.

A study of the accompanying tables shows that:

1. Kynurenic acid is not utilized by the body of the dog but is eliminated in the urine in an unchanged condition.

2. There is no simple relation between the amount of tryptophane administered and the kynurenic acid eliminated in the urine.

If the tryptophane were completely changed into kynurenic acid the proportion should be: 1 gram of tryptophane: 0.95 gram of kynurenic acid.

The greatest amount of kynurenic acid excreted by any of the dogs was only 38 per cent of the theoretical amount possible; the average was about 10 per cent and in some cases it was as low as or even lower than 5 per cent of the theoretical amount.

3. In each animal the excretion of kynurenic acid reached a maximum beyond which further increase in the dose of tryptophane was without effect.

The amount of tryptophane excreted by any one dog may depend upon the relative size of its liver. Thus a dog of 23½ pounds' weight excreted as much as 1.8 grams of kynurenic acid; a dog of 13½ pounds' weight excreted 0.64 gram of the acid; puppies of 6 pounds 14 ounces', and 4 pounds 5½ ounces' weight respectively excreted 0.3 and 0.13 gram of the acid on the same weights of tryptophane, etc.

4. The young animals have a greater capacity for the utilization of tryptophane than the fully grown dog has (Tables I c and II b).

5. There is no marked increase in the output of nitrogen during the tryptophane feeding period. The administration of the substance has therefore no marked effect on the general metabolic processes of the body (Table II c).

6. Even after flooding the digestive organs with a large dose of tryptophane, the corresponding amount of kynurenic acid was not eliminated; neither could any unchanged tryptophane be detected in the urine, nor could any other indol derivatives be detected in the urine by colorimetric methods (Table III).

SUMMARY.

The results of the investigation seem to indicate that:

1. Kynurenic acid apparently is not readily capable of further utilization in the body of the dog as all which is administered is excreted in the urine. It is therefore justifiable to assume that all the tryptophane converted into kynurenic acid in the body is excreted in the urine as such.

2. The production of kynurenic acid from tryptophane does not represent either the only or the main line of breakdown of tryptophane in the animal body; this is specially true of the young animal.

The evidence adduced in this paper seems to indicate that a certain amount of the tryptophane administered becomes "sidetracked" into kynurenic acid while the remainder is utilized in some direction not yet elucidated, but which may probably have some intimate connection with the furnishing of the particular substance or substances necessary for the maintenance of life of the individual.

The work described in this paper was undertaken originally at the suggestion of Dr. Hopkins, Cambridge, England. The author wishes to express her appreciation of the help he has given and the interest he has shown during the progress of the investigation.

TABLE I a.

The Repeated Administration of Tryptophane.
Dog A. (Weight 13½ lbs.)

Date.	Diet.	Volume of urine.	Kynurenic acid in urine estimated by Capaldi's method.
		cc.	*gm.*
Mar. 31– Apr. 1	250 gm. lean meat	190	
Apr. 1– 2	“ “ “ “	170	Traces.
2– 3	500 “ “ “	Not measured	
3– 4	500 gm. meat, 3 gm. tryptophane	400	0.648
4– 5	“ “ “ “ “ “	475	0.512
5– 6	“ “ “ “ “ “	253	0.578
6– 7	“ “ “ “ “ “	300	0.640
7– 8	“ “ “ “ “ “	339	0.625
8– 9	“ “ “ “ “ “	250	0.540
9–10	“ “ “ “ “ “	350	0.518
10–11	“ “ “ “ “ “	400	0.542
11–12	“ “ “ “ “ “	310	0.535
12–13	500 gm. lean meat	350	0.1403
13–14	“ “ “ “	440	0.0877
14–15	“ “ “ “	500	0.2025
15–16	“ “ “ “	Not estimated	
16–17	“ “ “ “	370	0.1072
17–18	“ “ “ “	290	0.0920
18–19	“ “ “ “	390	Spoilt.
19–20	“ “ “ “	360	0.0982

It was shown afterwards that the precipitates weighed as kynurenic acid between the dates April 12–20 were practically pure uric acid.

TABLE Ib

The Repeated Administration of Tryptophane to a Dog on a Meat Diet. Dog B. (Weight 23½ lbs.) Estimation in the Urine (1) of the Kynurenic Acid by Capaldi's Method. (2) Estimation of the Uric Acid Contaminating the Capaldi Precipitates and Hence the True Value for the Kynurenic Acid Output. (3) Estimation of the Uric Acid Content of the Urine by Hopkins' Method.

Date.	Diet.	Total volume of urine.	(1) Weight of *kynurenic acid* by Capaldi's method. *Pptes. A.* - kynurenic — uric acid.		(2) Kynurenic acid after deducting uric acid contaminating Pptes A.		(3) Total amount of *uric acid* in the urine estimated by Hopkins' method on 100 cc. of the urine.	
			(a) per cc.	(b) Total.	(a) per cc.	(b) Total.	(a) per cc.	(b) Total.
		cc.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
May								
21–22	500 gm. meat	288	0.000344	0.099	0.000277	0.080	0.000472	0.136
22–23	" "	380	0.000934	0.355	0.000724	0.275	0.000482	0.183
23–24	" "	340	0.00168	0.502	0.00130	0.441	0.000483	0.164
24–25	" "	544	0.00106	0.578	0.000901	0.491	0.000404	0.220
25–26	" "	464	0.00094	0.436	0.000728	0.338	0.000342	0.168
26–27	500 gm. meat and 5 gm. tryptophane	282	0.00218	0.707	0.00226	0.636	0.00056	0.155
27–28	" " " " "	349	0.00261	0.901	0.00300	0.832	0.000535	0.187
28–29	" " " " "	558	0.00349	1.946	0.00307	1.812	0.000571	0.319
29–30	" " " " "	322	0.00498	1.603	0.00487	1.573	0.000639	0.206
30–31	" " " " "	818	0.00221	1.804	0.00209	1.712	0.000309	0.253
June								
1–2	500 gm. meat	384	0.000810	0.247	Spoilt		0.000560	0.215
2–3	" "	366	0.000752	0.275	0.000511	0.187	0.000441	0.161
3–4	" "	316	0.000538	0.170	0.000332	0.105	0.000371	0.117
4–5	" "	461	0.000455	0.210	0.000276	0.127	0.000324	0.149
5–6	" "	338	0.000462	0.150	0.000248	0.0838	0.000426	0.143
			Mixed precipitates A (Capaldi)		*True value for kynurenic acid.*			

TABLE I c.

The Administration of Tryptophane to Puppies from the Same Litter, Aged 2 Months. Dogs C and D.

Date.	Diet.	Dog C. Weight 4 lbs 5½ oz		Dog D. Weight 6 lbs 14 oz.	
		Volume of urine	Kynurenic acid excreted	Volume of urine.	Kynurenic acid excreted
		cc.	*gm.*	*cc.*	*gm*
July 22-23	Bread and milk		Traces		Traces.
23-24	" " "		"		"
24-25	" " "		"		"
25-26	Bread and milk and 3 gm. tryptophane	290	0.0885	204	0.170
26-27	" " " " " "	136	0.158	132	0.255
27-28	" " " " " "	152	0.148	160	0.283
28-29	" " " " " "	170	0.135	220	0.316
29-30	" " " " " "	166	0.131	202	0.292
30-31	" " " " " "	144	0.125	130	0.253
July 31-Aug. 1	" " " " " "		Traces		Traces.
Aug. 1-2	" " " " " "		Nil		Nil.

It was ascertained that on a diet of bread and milk there was no excretion of uric acid.

TABLE II a.

The Administration of Increasing Doses of Tryptophane to a Dog. Dog B. (Weight 23½ lbs.)

Date.	Diet.	Volume of urine.	Uric acid excreted.	Kynurenic acid excreted.
		cc.		*gm.*
Nov.				
18–19	Bread and milk and 50 gm. meat		Traces	Traces.
19–20	" " " " " " "		"	"
20–21	" " " " " " "	295	"	"
21–22	The same and 1 gm. tryptophane	305	"	0.0939
22–23	" " " " " "	None		
23–24	The same and 2 gm. tryptophane	560	"	0.156
24–25	" " " " " "	345	"	0.354
25–26	" " " 4 " "	None		
26–27	" " " 6 " "	180	"	0.224
27–28	" " " " " "	500	"	0.679
28 29	Dog died			

A post mortem examination of the dog showed considerable hemolysis of the liver and kidney.

TABLE II b.

The Administration of Increasing Doses of Tryptophane to a Puppy Aged 5 Months. Dog C. (Weight 16 lbs.)

Date.	Diet.	Volume of urine.	Uric acid excreted.	Kynurenic acid excreted.
		cc.		*gm.*
Oct.				
27–28	Bread 150 gm., milk 250 cc., meat 25 gm.		Nil	Traces.
28–29	" " " " " " " " "		"	"
29–30	" " " " " " " " "		"	"
30–31	" " " " " " " " "		"	"
Oct. 31–Nov. 1	The same and 1 gm. tryptophane	380	"	Spoilt.
1– 2	" " " 2 " "	255	"	0.1326
2– 3	" " " 4 " "	406	"	0.0854
3– 4	" " " 6 " "	134	"	0.0670
4– 5	" " " 8 " "	260	"	0.0359
5– 6	Bread, milk, and meat	390	"	0.0156

TABLE II c.

Administration of Increasing Doses of Tryptophane. Estimation of the Total Output of Kynurenic Acid and of Nitrogen during the 24 Hour Periods. Dog F. (Weight 15 lbs.)

Date.	Diet.	Volume of urine.	Kynurenic acid excreted.	Total nitrogen (Kjeldahl).	Uric acid excreted.
		cc.	*gm.*	*gm.*	
Feb. 27–Mar. 4	150 gm. dog biscuits and 25 gm. meat daily		Not estimated		Nil.
Mar.					
4– 5	150 gm. biscuits and 25 gm. meat	132	0.0134	4.03	"
5– 6	" " " " "	128	0.0110	3.56	"
6– 7	" " " " "	218	0.100	5.21	"
7– 8	The same and 1 gm. tryptophane	188	0.0438	4.03	"
8– 9	" " " 2 " "	149	0.1880	4.40	"
9–10	" " " 3 " "	183	0.1994	4.17	"
10–11	" " " 4 " "	256	0.1736	4.05	"
11–12	" " " 5 " "	143	0.4332	4.16	"
12–13	" " " 6 " "	221	0.3038	5.20	"
13–14	" " " 7 " "	169	0.4120	4.46	"
14–15	150 gm. dog biscuits and 25 gm. meat	183	0.0048	4.58	"
15–16	" " " " " "	147	0.0078	5.06	"
16–17	" " " " " "	103	0.0060	2.38	"
17–18	" " " " " "	174	0.0074	4.71	"

There was no free tryptophane in the urine and no indication of the elimination of indol pigments.

1 gm. of kynurenic acid contains 0.074 gm. of nitrogen.
1 " " tryptophane " 0.134 " " "

TABLE III.

The Administration of Large Doses of Tryptophane to a Dog. Dog B. (Weight 23½ lbs.)

Date.	Diet.	Volume of urine.	Kynurenic acid excreted.
		cc.	*gm*
Oct.			
13–14	Bread 250 gm., milk 250 cc., meat 50 gm.	540	Traces.
14–15	“ “ “ “ “ “ “ “ “	342	“
15–16	The same and 10 gm. tryptophane (Only about ⅓ of food eaten.)	66	0.212
16–17	The same and 10 gm. tryptophane	685	1.256

A further dose of 10 gm. of tryptophane was given with the dog's food on Oct. 17 but by this time the dog refused to touch any more food in the cage whether with or without the addition of tryptophane.

Tryptophane has a very bitter taste and it was difficult to administer as much as 10 gm. at a time and successfully mask its bitterness.

TABLE IV a.

The Excretion of Uric Acid by a Dog on a Meat Diet. (Compare with Results for Dog B, Table I b.) Dog H.

Date.	Diet.	Volume of Urine.	Total output of uric acid for the 24 hours estimated by	
			Folin's method on 5 cc. urine.	Hopkins' method on 100 cc. urine.
		cc.	*gm.*	*gm.*
Apr.				
1– 2	Biscuits	150	Traces	
2– 3	“	235	“	
3– 4	1 lb. lean meat	167	0.0729	
4– 5	“ “ “ “	264	0.131	
5– 6	“ “ “ “	160	0.0929	
6– 7	“ “ “ “	150	0.0622	
7– 8	“ “ “ “	175	0.155	0.160
8– 9	“ “ “ “	324	0.239	0.250
9–10	“ “ “ “	232	0.130	
10–11	Biscuits	52	0.0150	
11–12	“	160	Traces	

TABLE IV b.

The Excretion of Uric Acid by Dogs on Diets with Low Purine Value.

Date.	Diet	Uric acid excreted.	Kynurenic acid excreted.
Dog A on diet of bread and milk for some days previous.			
June			
13–14	Bread and milk	Traces	0.0148
14–15	“ “ “	“	0.0083
15–16	“ “ “	“	Traces.
Dog B on diet of bread and milk and small amount of meat for some days previous.			
Nov.			
18–19	Bread, milk, and 50 gm. meat	Traces	Traces.
19–20	“ “ “ “ “ “	“	“
20–21	“ “ “ “ “ “	“	“
Dog E on diet of dog biscuits for some days previous.			
Dec.			
16–17	150 gm. dog biscuits	Traces	Traces.
17–18	“ “ “ “	“	“
18–19	“ “ “ “	“	“
Dog F on diet of dog biscuits and small amount of meat.			
Mar.			
4–5	150 gm. biscuits and 25 gm. meat	Traces	0.0134
5–6	“ “ “ “ “ “ “	“	0.0110
6–7	“ “ “ “ “ “ “	“	0.0110

TABLE IV c.

The Excretion of Uric Acid by a Dog Kept Without Food from Feb. 24 to Mar. 4. Dog G.

Date.	Volume of urine.	Total excretion of uric acid (estimated by Folin's method on 5 cc. of urine).
	cc.	*gm.*
Feb.		
24–25	410	0.013
25–26	516	0.019
26–27	344	0.030
27–28	710	0.032
Feb. 28–Mar. 1	260	0.031
Mar.		
1–2	452	0.023
2–3	398	None.
3–4	276	None.

THE WAVERLY PRESS
BALTIMORE, U. S. A.

394

Reprinted from The Canadian Medical Association Journal,
January, 1913

A CASE OF HÆMOLYTIC JAUNDICE WITH SPLENOMEGALY

By Fletcher McPhedran, B.A., M.B.
Demonstrator in Clinical Medicine

and Harold Orr, M.B.
Fellow in Pathological Chemistry, the University of Toronto

MINKOWSKI and Bettman described, in 1900, a series of cases of jaundice presenting several interesting features. The patients were hardly ever affected by the jaundice. There was no bradycardia, no irritation of the skin, and no xantheloma. The tint was unmistakable and increased after exertion, cold baths, or even after fits of anger. Definite pathological changes were to be found in certain organs; the spleen was enlarged and showed hyperplasia and hyperæmia. The liver was somewhat enlarged, but no special changes were found in it, and the bile passages were quite clear. The kidneys were marked by a siderosis similar to that found in pernicious anæmia. This pigment was later found to be iron and to be confined to the kidneys. The blood picture was that of a secondary anæmia without nucleated red cells, but with polychromatophilia, and with a fairly large number of large red cells which were taken to be quite young.

Minkowski considered that the whole process depended upon a primary lesion in the spleen; that the blood cells were destroyed there, and that the jaundice came from the increased pigments in the serum arising from the great blood destruction. Somewhat later Chauffard observed that the red blood cells of individuals suffering from this malady were not so resistant to hypotonic sodium chloride solutions as were those of the normal individual. If the red blood cells of a normal individual are washed free from serum and dropped into a series of tubes containing progressively diminishing percentages of NaCl,—say from that of normal saline ·85 per cent. down to ·80 per cent., ·78 per cent., ·76 per cent., ·74 per cent. and so on down to nothing—it will be found that the normal cells show no trace of hæmolysis until ·42 per cent. of NaCl is reached, and that there will not be complete destruction of the cells until about ·18 per cent. In this condition, however, it was observed that the cells so treated commenced to hæmolyse at ·66 per cent., or even higher, and that the stage of complete hæmolysis was reached at about ·34 per cent. Further, the same writer noted that if a stain, such as Unna's polychrome methylene blue, were dropped on to the skin, and the blood obtained from a prick through the drop, so that the cells might be stained without fixation, many of the cells showed a curious reticular granulation. Normally this is seen in 1 to 2 per cent. of blood, but in this condition and in infants' blood, and in blood after severe hæmorrhages, the percentage may rise to as high as 10 or even 40 per cent. Vaughan, who first described this finding, thought the presence of so many such cells a sign of new blood, and the majority of investigators have agreed with him.

The urine is always highly coloured from the presence of large amounts of urobilin, but never contains bile pigments. The stools are normal in colour. The blood is characterized by a slight leucocytosis, and by an anæmia that varies and upon which the patient's symptoms generally depend. They are also frequently subject to attacks of gall-stone colic, though many have been operated upon without stones being found. Many are well, "being icterics rather than patients," as Chauffard says, and none have ever complained of the bradycardia, pruritus, or tendency to hæmorrhages so commonly found in jaundice.

Recently we had the opportunity of seeing a well-marked case of this malady. A. B., aged thirty, white, male, settlement worker, complained of pain in the right scapular region, jaundice, headache, and constipation, all of which have dated more or less from his early childhood. His father died at the age of sixty from some acute illness, and he had been yellow all his life. One half-brother on the paternal side was always very yellow and died in India at an early age. The patient has never had any acute illness since childhood. He has never had typhoid fever, pneumonia, or malaria, nor has he ever lived in any locality where malaria is endemic. Once, about five years ago, he had pruritus, when he remembers that the jaundice was severe. He has never had any other skin trouble, he has no tendency to hæmorrhages from the stomach or elsewhere, and his pulse is always about seventy. About the same time he was told that his spleen was large. He had felt full there and thinks that the size of the spleen has not changed. He is a pale, rather nervous man, with quite marked jaundice in the skin, conjunctivæ, and palate. The eyes are prominent, but there is no further sign of hyperthyroidism. The tongue is pale and somewhat frothy. The throat, chest, glands and joints are normal. There is no undue pigmentation of the skin. The abdomen is large, pendulous, rather lacking in tone. It moves freely in respiration. On palpation there is some tenderness over the left side generally. The spleen is much enlarged, reaching to the right of and below the umbilicus. It is firm, smooth, and not tender on deep palpation, and moves freely on deep respiration. The liver reaches from the sixth interspace to two finger breadths below the costal margin in the right nipple line. It is firm, smooth, and not tender. The stomach is not abnormal. The reflexes are normal.

The blood flowed freely, clotted in about normal time, and there was about 70 per cent. hæmoglobin (Dare). The white blood cells numbered nine thousand, the reds three million five hundred thousand to the cmm. In the fresh preparations, the reds were about normal in size, a few were larger and all looked rather pale. Stained by Hasting's stain, the whites showed no marked abnormality; there were no young forms. No nucleated reds were found, but there was quite evident polychromatophilia. When Unna's stain was used, a slight increase in the number of reticulated cells as compared to the normal, was found. When the washed corpuscles were added to hypotonic salt solutions, it was found that there was initial hæmolysis at ·56 per cent. and complete at ·36 per cent. The stools were dark in colour, not increased in volume, and showed no peculiarity. The urine passed per day was about normal in amount, dark in colour, acid, sp. gr. 1025, and contained no albumen, or sugar, or bile pigment. With zinc acetate and ammonia, there was an intense greenish fluorescence, and the spectroscope showed the test for urobilin.

An attempt was made to estimate the amount of urobilin passed in the urine and fæces in one day by the method employed by Simpson.[1] The urine collected for twenty-four hours was treated with dilute sulphuric acid and exposed to the light for some time to convert the urobilinogen into urobilin. It was then saturated with ammonium sulphate, and the precipitate filtered off and extracted with chloroform. After evaporating off the chloroform the remainder was taken up in water, reprecipitated with ammonium sulphate, again extracted with chloroform, and this series of operations repeated a third time. The residue left after evaporating the final chloroform solution weighed 66 mgms. A solution of this preparation containing 16·1 mgms. in one litre just showed the urobilin spectrum to be seen when examined in a layer 15 mm. thick with a Schmidt Haensch pocket spectroscope with scale and electric illumination. (Catal. D No. 230.) The fæces passed on the same day, the bowels having been opened regularly for some days previously once a day, were mixed with water, acidified with sulphuric acid, and allowed to stand exposed to the light. The mixture was then filtered, and further extraction of the solid material proceeded with till, on filtration, the extract was practically colourless. The mixed filtrates measured three litres, and a portion diluted twenty times still just showed the spectrum of urobilin to be seen in a layer of 15 mm. thick.

Whereas, therefore, the preparation from the urine could be diluted to four litres and still show the urobilin spectrum under the standard conditions observed, that from the fæces could be diluted to sixty litres; there was thus found fifteen times as much urobilin in the fæces as in the urine. Simpson, observing similar conditions, found that this dilution corresponds to a solution of 10 mgms. in one litre of a preparation of pure urobilin prepared according to the method of Garrod and Hopkins, and calculated the amount of urobilin in various samples of urine and fæces on this basis. Calculating similarly from our data, namely, that under the conditions that we observed, the standard dilution of urobilin contained 16 mgms. in one litre of a preparation of urobilin prepared from urine as described above, we should arrive at the result that the urine contained 66 mgms. of urobilin, and the fæces 996, and that the total excretion of urobilin amounted to 1·05 grms. in the twenty-four hours. But apart from the fact that the preparation of urobilin that we obtained from the urine was not prepared strictly according to the method of Garrod and Hopkins, even if it had been, it is doubtful, in the light of the work of Hans Fischer,[2] whether quantitative estimations of urobilin can be based on such preparations at all. However, comparative results may be obtained, and the urobilin excretion in this case may be compared with that in those examined by Simpson. We took the twenty-four hour specimens of urine and fæces from a normal adult and treated them in the same manner as the specimens from A. B. were treated. No absorption bands in the spectrum for urobilin could be seen in the urine extract, at any dilution, but when the fæcal extract was diluted up to ten litres, there was still a faint absorption to be seen under the standard conditions related above. That is to say, there was at least six times as much urobilin in the extract of the patient as in that of the control normal adult. From such a comparison it is clear that the excretion was very high, and the amount of blood pigment destroyed must have formed a considerable proportion of the whole, considering that the total amount of hæmatin in the body cannot be much more than thirty grams.

It is interesting to note that notwithstanding this great destruction of red blood cells, as evidenced by the urobilin excretion, the blood shows in the Romanowsky and Unna preparations very slight evidences of active regeneration. Luzzatto and Ravenna[3] have shown that while the presence of polychromatophilia and of nucleated red cells in the blood is no evidence of the degree of the severity of the anæmia, still it shows increased activities of the marrow and of the hæmatopoietic cells. Here is a case where the loss of blood must have been excessive for a long period, and yet the cells morphologically show little change. Banti[4] thinks that the cause of the trouble lies in the fact that the spleen not only has its normal function of destruction of the red blood cells considerably raised, but that it has the added faculty of reducing the resistance of the cells to hypotonic salt solutions. Lintvarev[5] has recently described a similar case wherein he found, in the spleen and in the liver tissue, a large number of cells that were evidently destroying the red cells. They were about ten or twelve times as big as a red cell, of a plastic viscid protoplasm, and filled with red cells undergoing destruction. He and Dehn[6] thought that all cases of hæmolytic jaundice could be explained by the presence of these cells, but no other writer has reported their presence in any of his cases. Dehn further considered that these cases would be much benefited by *x*-ray treatment acting upon the lymphoid tissues which he thought hypertrophied, and showed that splenectomized dogs withstood the action of hæmolytic poisons much better than normal dogs. Pel[7] has recently shown that while the resistance of the dog's red cells to hypotonic salt solutions is the same as in man, it rises after splenectomy so that the first trace of hæmolysis is at ·35 per cent. instead of ·42 per cent. This increased resistance remains constant for over a year, it is not explainable by any change in the serum, and no difference could be shown in the red or white cells after splenectomy.

Recently we have had the opportunity of investigating the blood of two patients who had splenectomy done. One, a man of forty-five years, had his spleen removed eighteen months ago for splenic anæmia of the Gaucher type; the other, a man of eighteen, had his excised four months ago owing to a severe kick from a horse; In the latter, there was no change in the resistance of the red cells.

in the former, there was initial hæmolysis at ·40 per cent., which must be considered well within the laboratory error. In neither case was there any excess of granulated cells when stained by Unna's stain. Banti (l.c.) has reported several cases treated by splenectomy. One patient operated upon in 1903 has remained healthy ever since. The spleen showed none of the large cells considered so important by Lintvarev and Dehn, but only hyperplasia, hyperæmia, and marked phagocytosis of the red cells in the pulp and sinuses, by cells found normally in the spleen. Other cases related by Banti[8] and by Roth[9] have been equally successful. In the latter's case the patient was still slightly icteric and the resistance of the reds still low—there was initial hæmolysis at ·58 per cent. sodium chloride; but he had no anæmia and could work hard in spite of some epileptic attacks from which he had long suffered.

It will be seen that the symptoms are those of anæmia, and that the predominating clinical signs in the common case inconvenience the patient little or not at all. While no definite ætiology can be found, there seems little doubt that the primary cause of trouble is in the spleen, though there is no evidence to show that there is any specific hæmolysin there. Guizzetti[10] has recently reported on a large family of persons afflicted with this anomaly; there were two deaths with autopsy and signs of congenital syphilis. The spleens, while large, were not nearly so large as those found in almost all other spleen affections. Probably the jaundice is due to the deposits of urobilin in the tissues, for the serum is yellow, and while bile pigments have not been found in the serum, urobilin has been found there several times. Further, there is no reason to believe that the liver is producing an excess of bile or that there is any absorption by the capillaries. The skin affections of itching and xanthoma and the bradycardia are evidences of bile in the serum, but they are transitory and often not present when the jaundice is at its worst.

If it is admitted that the spleen is the source of the trouble the outlook is better for those severely affected, as was our patient. There are methods of altering the resistance of the blood cells in animals with cholesterin or saponin, but these methods so far are inapplicable to man. But the spleen can be safely and easily removed and the great destruction of cells stopped or lessened.

We take pleasure in thanking Professor Alexander McPhedran for the opportunity of seeing this case and the case of splenic anæmia; Dr. John R. Parry, of Hamilton, for seeing his patient with the excision of the spleen following injury; and Professor J. B. Leathes for advice and help on many occasions.

References:—

A good bibliography is given by Thayer and Morris, Johns Hopkins Hospital, 1911, xxii, 95.

1. Simpson, *Biochem. Journ.*, 1910, v, 378.
2. Fischer, *Zeitschrift f. Physiol. Ch.*, 1911, lxxiii, 204 and 271.
3. Luzzatto and Ravenna, quoted, *Zentralblatt f. gesamte inn. Med.*, 1912, iii, 271.
4. Banti, quoted, *Zentralblatt*, 1912, ii. 46.
5. Lintvarev, *Ann. de l'Inst. Pasteur*, 1912, xxvi, 51.
6. Dehn, quoted, *Zentralblatt, f.g. inn. Med.*, 1912, iii, 99.
7. Pel, *Deutsch. Arch. f. klin. Med.* 1912, cvi, 392.
8. Banti, quoted, *Zentralblatt, f.g. inn. Med.*, 1912, ii, 650.
9. Roth, *Zeitscrift f. klin. Med.*, 1912, lxxvii, 23.
10. Guizzetti, "Ziegler's Beiträge," 1912, lii, 15.

395

UNIVERSITY OF TORONTO STUDIES

MEDICAL RESEARCH FUND

No. 1: ON THE HAEMOLYTIC PROPERTIES OF FATTY ACIDS AND THEIR RELATION TO THE CAUSATION OF TOXIC HAEMOLYSIS AND PERNICIOUS ANAEMIA, by W. F. McPhedran

(Reprinted from the Journal of Experimental Medicine, Vol. XVIII)

No. 2: BIOLOGICAL CURVES OBTAINED DURING THE ONSET AND COURSE OF TUBERCULOUS INFECTION, by A. H. Caulfeild and F. S. Minns

(Reprinted from the Transactions of the Tenth Annual Meeting of the National Association for the Study and Prevention of Tuberculosis)

No. 3: TUBERCULIN TREATMENT BASED UPON CLINICAL AND BIOLOGICAL DATA, by A. H. Caulfeild and F. S. Minns

(Ibid.)

No. 4: THE EXCRETION OF NITROGEN IN FEVER, by N. C. Sharpe and K. M. B. Simon

(Reprinted from the Journal of Experimental Medicine, Vol. XX)

No. 5: ON FATTY CHANGES IN THE LIVER, HEART AND KIDNEY, by C. G. Imrie

(Reprinted from the Journal of Pathology and Bacteriology, Vol. XIX)

No. 6: ON THE FAT IN THE BLOOD IN A CASE OF LIPAEMIA, by C. G. Imrie

(Reprinted from The Journal of Biological Chemistry, Vol. XX)

THE UNIVERSITY LIBRARY: PUBLISHED BY
THE LIBRARIAN, 1915

University of Toronto Studies
COMMITTEE OF MANAGEMENT

Chairman: Robert Alexander Falconer, M.A., Litt.D., LL.D., D.D.
President of the University

Professor W. J. Alexander, Ph.D.
Professor W. H. Ellis, M.A., M.B.
Professor J. J. Mackenzie, B.A.
Professor J. P. McMurrich, Ph.D.
Professor G. H. Needler, Ph.D.
Professor George M. Wrong, M.A.

General Editor: H. H. Langton, M.A.
Librarian of the University

[Reprinted from the JOURNAL OF EXPERIMENTAL MEDICINE, Vol XVIII, No. 5 1913.]

ON THE HEMOLYTIC PROPERTIES OF FATTY ACIDS AND THEIR RELATION TO THE CAUSATION OF TOXIC HEMOLYSIS AND PERNICIOUS ANEMIA.*

BY WILLIAM FLETCHER McPHEDRAN, M.B.

(From the Department of Pathological Chemistry of the University of Toronto, Toronto.)

In the many attempts that have been made to interpret the phenomena of pernicious anemia by the supposition that a powerful hemolytic agent is at large in the body, the fact that substances soluble in alcohol or ether may have strong hemolytic properties has been much insisted upon. More especially attention has been directed to fatty acids and their alkaline salts, and since the work of Faust and Tallquist (1), in which the anemia caused by *Bothriocephalus latus* was ascribed to the unsaturated acid, oleic acid, which the worm contains, there has been a tendency to regard particularly the unsaturated fatty acids as playing possibly an important part in other forms of anemia. The marked difference described by Faust and Tallquist between the hemolytic properties of oleic acid and its salts on the one hand, and stearic acid and its salts on the other, which was also observed in the comparison of other pairs of acids standing to each other in the same relation as these two, seemed to indicate that the lytic power of oleic acid might reside in the one chemical point of difference between it and stearic acid; namely, the pair of doubly united carbon atoms present in its molecule.

In recent years we have come to recognize that oleic acid is not the only unsaturated fatty acid that occurs in the body. We have clear evidence that in animal cells, with probably the single exception of the adipose connective tissue corpuscles in which the crude food fat is stored after absorption if not needed for immediate use, there are considerable quantities of fatty acids in which more than one such pair of doubly united carbon atoms occur in each molecule. If, therefore, in oleic acid the single pair of unsaturated atoms gives

---

* Received for publication, June 2, 1913.

rise to such remarkable properties in relation to red blood corpuscles, it seemed possible that the hemolytic properties of the fatty acids more unsaturated than oleic acid, with two, three, or even four, such pairs of doubly united carbon atoms, might be far greater still. And if this were so, a new direction might be given to attempts such as those of Joannovics and Pick (2) to look for the cause of toxic anemias in the fatty products liberated from cells destroyed by the action of a toxic principle.

In order to test this theory it was necessary in the first instance to fix some standard of hemolytic power with which to compare the properties of any substance to be investigated. For this purpose 0.2539 of a gram of oleic acid (Schuchardt's acidum oleinicum purissimum), which was found to have almost the correct molecular weight, 285, and almost the theoretical iodine value (calculated 90.0; observed 87.5), was dissolved in 100 cubic centimeters of methyl alcohol and neutralized with the calculated amount of standard soda solution; and immediately before each experiment one cubic centimeter of this solution was diluted with normal saline and the resulting emulsion used. In all the experiments the same amount of twice washed red blood corpuscles was used, 0.5 of a cubic centimeter of a 5 per cent. suspension; except where expressly mentioned the corpuscles were those of the sheep; and the volume was made up with 0.9 per cent. salt solution in every case to two cubic centimeters. The tubes were shaken, kept at 37° C. for two hours, and the results read then and again after standing for twenty-four hours at room temperature. Table I shows the result on one of many occasions on which the amount of the sodium salt of this oleic acid that was necessary to give complete hemolysis was determined and found to be the same.

TABLE I.

Sodium Oleate in Methyl Alcohol (1 c.c. = 2.75 mg.) Diluted 1 to 20 with Normal Saline (1 c.c. = 0.137 mg.).

Tube.	Lytic agent.	Result.
1	0.1 c.c.	Partial.
2	0.2 c.c. = 0.027 mg.	Partial, almost complete.
3	0.3 c.c. = 0.041 mg.	Complete.
4	0.4 c.c.	Complete.

In order to compare these results with those obtained by other workers, and also with some of those to be described in which human blood corpuscles were employed, on more than one occasion the effect of the same preparation on the blood of other species of animals was determined, and from the results given in table II it is apparent that no difference could be detected in the amounts necessary for complete hemolysis of equal quantities of corpuscles from the sheep, rabbit, ox, dog, and man, for all of which species the minimum hemolytic dose of sodium oleate is about 0.03 of a milligram for 0.5 of a cubic centimeter of a 5 per cent. suspension of corpuscles.

TABLE II.

Sodium Oleate in Methyl Alcohol (1 c.c. = 2.75 mg.) Diluted 1 to 80 with Normal Saline (1 c.c. = 0.034 mg.).

Tube	Lytic agent.	Result.				
		Dog.	Rabbit.	Sheep.	Ox.	Man.
1	0.3 c.c.	Trace	Partial	Trace	Trace	Partial.
2	0.4 c.c.	Partial	Partial	Trace	Trace	Partial.
3	0.8 c.c. = 0.027 mg.	Complete	Complete	Complete	Complete	Complete.
4	1.2 c.c. = 0.039 mg.	Complete	Complete	Complete	Complete	Complete.

We can now compare with this result those obtained by others. Noguchi determined the minimum lytic dose of sodium oleate for 2 c.c. of a 5 per cent. suspension of ox corpuscles to be 0.07 mg.; for the quantity of sheep's corpuscles, 0.5 c.c., used in the experiments here reported, the dose according to Noguchi (3) would be therefore 0.018 mg. Meyerstein (4), who also made careful determinations of the amount of a 0.05 per cent. sodium oleate solution necessary completely to hemolyze 1 c.c. of a 5 per cent. suspension of sheep's corpuscles gives as his result 0.1 c.c., or 0.05 mg.; for 0.5 c.c., therefore, 0.025 mg. Finally from Lamar's (5) results the lytic dose of the same substance for the same amount of corpuscles may be calculated and with the same result. There is thus a fair agreement between these sets of observations.

Noguchi	0.018 mg.
Meyerstein	0.025 mg.
Lamar	0.025 mg.
McPhedran	0.027 mg.

It is therefore clear that the standard for hemolytic activity here adopted is fairly well defined, that between 0.02 and 0.03 of a milligram of sodium oleate will completely lake 0.5 of a cubic centimeter of a 5 per cent. suspension of red blood corpuscles in the hands of different workers; or, in other words, one milligram is the lytic dose for one cubic centimeter of corpuscles of the rabbit, sheep, ox, dog, or man. When, therefore, a substance is spoken of as having intense or strong or weak hemolytic properties, which is commonly done without reference to any definitely fixed scale or standard, it would be far better if a simple comparison of the minimum hemolytic dose of the substance in question with that of sodium oleate were substituted for often misleading epithets used in designating its activity.

In the tables that follow the results are given of experiments in which a number of fatty acids have been tested out in this way.

Palmitic acid (Schuchardt's purissimum) (table III), as an example of a saturated fatty acid, and probably the one which is most abundant in food, was converted into sodium salt, and a solution in methyl alcohol of which one cubic centimeter contained 2.6 milligrams was prepared. Of this one cubic centimeter diluted ten times with warmed normal saline gave an emulsion with which complete hemolysis was not obtained with the largest quantity used. The minimum hemolytic dose of sodium palmitate is therefore more than 0.4 of a milligram, or more than twelve times as great as that of sodium oleate.

TABLE III.

Sodium Palmitate in Methyl Alcohol (1 c.c. = 2.6 mg.) Diluted 1 to 10 with Normal Saline.

Tube.	Lytic agent.	Result.
1	0.1 c.c.	Trace.
2	0.2 c.c.	Trace.
3	0.4 c.c.	Partial.
4	0.8 c.c.	Partial.
5	0.6 c.c. = 0.42 mg.	Partial.

TABLE IV.

Sodium Erucate in Methyl Alcohol (1 c.c. = 3.32 mg.) Diluted 1 to 10 with Normal Saline (1 c.c. = 0.33 mg.).

Tube.	Lytic agent.	Result.
1	0.1 c.c. = 0.033 mg.	Partial.
2	0.2 c.c. = 0.066 mg.	Complete.
3	0.25 c.c.	Complete.
4	0.3 c.c.	Complete.

TABLE V.

Sodium Linoleate in Methyl Alcohol (1 c.c. $= 2.5$ mg.) Diluted 1 to 80 with Normal Saline (1 c.c. $= 0.03$ mg.).

Tube.	Lytic agent.	Result.
1	0.2 c.c.	Partial.
2	0.4 c.c.	Partial.
3	0.8 c.c. = 0.025 mg.	Partial.
4	1.2 c.c. = 0.037 mg.	Complete.

Erucic acid (table IV), a commercial preparation (Schuchardt's), which was found to have the molecular weight 338 and iodine value 61.8 (calculated 340 and 74.6, respectively), was used in a similar way. The minimum lytic dose is about twice that of sodium oleate.

Linoleic acid is an acid that is more unsaturated than oleic acid. There being two pairs of doubly united carbon atoms in the molecule it was conceivable that the hemolytic power might be greater than that of oleic acid in which there is only one. A commercial preparation of this acid (Schuchardt's) was used; it is not probably possible to obtain strictly pure linoleic acid and this specimen, as was expected, gave neither the theoretical molecular weight nor the theoretical iodine value. But its iodine value was more than 186, and it therefore clearly contained a large amount of acid more unsaturated than oleic or erucic and serves as the first test of the theory that if it is the unsaturated carbon atoms that confer on these acids their hemolytic properties, the more of such atoms there are in an acid the more actively it should cause hemolysis.

The result of the experiment shown in table V, which was confirmed on other occasions, would show that the minimum hemolytic dose of the sodium salt of linoleic acid, lying as it does between 0.037 and 0.025 of a milligram, is not smaller than, but approximately the same as, that of the standard sodium oleate.

Lamar (5) has also compared the hemolytic power of linoleic acid and its potassium salt with that of oleic acid and sodium and potassium oleates. For sodium oleate, as pointed out above, our figures agree very closely, but he found linoleic acid to be more active than oleic, and its potassium salt to be three or four times as active as the oleates. This discrepancy is difficult to account for. But that the hemolytic power of unsaturated fatty acids is not related to the unsaturated carbon atoms in the way that his results would probably suggest will be clear from what follows.

The next point investigated was the hemolytic properties that result when the unsaturated carbon atoms of oleic acid become saturated by the introduction of chlorine, bromine, or iodine, or of hydroxyl groups. If the pronounced difference between the lytic power of oleic acid and the corresponding saturated acid, stearic acid, is due to the chemical activity of the unsaturated carbon atoms, we should expect this difference to disappear when the oleic acid is converted into dibromostearic, chloriodostearic, monobromostearic, or dihydroxystearic.

Dibromostearic acid was prepared in the usual way by treating oleic acid dissolved in glacial acetic acid with bromine dissolved in the same solvent as long as it was absorbed, and washing the product with water till all the acetic acid and uncombined bromine had been removed.

Chloriodostearic acid was obtained by treating oleic acid with excess of Wijs's solution of iodine chloride, as in determining the iodine value.

The monobromostearic was obtained by treating oleic acid with hydrobromic acid.

Another acid with the bromine in the α position was prepared by brominating stearic acid itself; the smallest quantity of this that caused complete hemolysis under the standard conditions was almost exactly the same as in the case of the first mentioned monobromostearic acid; viz., 0.036 of a milligram, and the minimum lytic dose lies between this value and 0.018.

As is apparent from tables VI to VIII, the three saturated halogen derivatives of oleic acid are none of them appreciably less actively hemolytic than oleic acid itself; although none of them

TABLE VI.

Potassium Dibromostearate in Methyl Alcohol (1 c.c. = 4 mg.) Diluted 1 to 80 with Normal Saline (1 c.c. = 0.05 mg.).

Tube.	Lytic agent.	Result.				
		Sheep.	Rabbit.	Man.		
				A.	*B.*	*C.*
1	0.3 c.c.	Partial	Partial	Trace	Partial	Trace.
2	0.4 c.c. = 0.02 mg.	Partial	Partial	Partial	Partial	Partial.
3	0.8 c.c. = 0.04 mg.	Complete	Complete	Partial	Almost complete	Complete.
4	1.2 c.c.	Complete	Complete	Complete	Complete	Complete.

TABLE VII.

Sodium Chloriodostearate in Methyl Alcohol (1 c.c. = 4 mg.) Diluted 1 to 80 with Normal Saline (1 c.c. = 0.05 mg.).

Tube	Lytic agent.	Result.
1	0.3 c.c.	Trace.
2	0.4 c.c. = 0.02 mg.	Almost complete.
3	0.8 c.c. = 0.04 mg.	Complete.
4	1.2 c.c.	Complete.

TABLE VIII.

Sodium Monobromostearate in Methyl Alcohol (1 c.c. = 3.27 mg.) Diluted 1 to 80 with Normal Saline (1 c.c. = 0.041 mg.).

Tube	Lytic agent.	Result.
1	0.3 c.c.	Partial.
2	0.4 c.c. = 0.016 mg.	Partial.
3	0.8 c.c. = 0.032 mg.	Complete.
4	1.2 c.c.	Complete.

contain any unsaturated carbon atoms, the lytic dose under the standard conditions adopted for this work is practically the same as that of oleate, in the neighborhood of 0.03 of a milligram. Indeed the volume of equimolecular solutions necessary for complete hemolysis is actually smaller in the case of the salts of these acids than in the case of sodium oleate. This disposes of the idea which has been current, that oleic acid is the potent hemolytic agent that it is on account of the chemical reactivity of the unsaturated carbon atoms that alone distinguish it chemically from stearic acid. The saturation of these atoms with halogens leaves the molecule as strongly hemolytic as before.

On the other hand, the introduction of two hydroxyl groups where there are two vacant carbon bonds in oleic acid at once reduces the hemolytic power, at least as much as the two hydrogen atoms that in stearic acid have taken these positions. The product dioxystearic acid is a solid with a relatively high melting point, and it is easy to suggest that the change thus effected in the hemolytic properties of the molecule is the result of the change in its physical nature; at any rate the loss of hemolytic power is not due to the loss of the chemical properties of the unsaturated carbon atoms. The preparation of dioxystearic acid employed was made from oleic acid obtained from the pig's liver by oxidizing it in the cold with alkaline permanganate; its preparation and properties are described by Hartley (6) to whom I am indebted for the use of his specimen.

So far the tests described above have been carried out with pure or practically pure single substances. Tables X and XI contain the results of other experiments in which the theory is tested that if unsaturated fatty acids are hemolytic because they are unsaturated, the more unsaturated they are the greater their hemolytic activity should be.

TABLE IX.

Sodium Dihydroxystearate in Methyl Alcohol (1 c.c. = 3 mg.).

Tube.	Lytic agent.	Result.
1	0.05 c.c.	Partial.
2	0.1 c.c.	Partial.
3	0.2 c.c.	Partial.
4	0.4 c.c.	Partial.
5	0.8 c.c. = 2.4 mg.	Partial.

TABLE X.

Sodium Soaps of Mixed Fatty Acids Obtained from Cod Liver Oil Dissolved in Methyl Alcohol (1 c.c. = 2.53 mg.) Diluted 1 to 80 with Normal Saline (1 c.c. = 0.03 mg.).

Tube.	Lytic agent.	Result.
1	0.3 c.c.	Partial.
2	0.4 c.c. = 0.012 mg.	Partial.
3	0.8 c.c. = 0.024 mg.	Complete.
4	1.2 c.c.	Complete.

TABLE XI.

Sodium Soaps of Mixed Fatty Acids Obtained from Linseed Oil Dissolved in Methyl Alcohol (1 c.c. = 2.78 mg.) Diluted 1 to 80 with Normal Saline (1 c.c. = 0.03 mg.).

Tube.	Lytic agent.	Result.
1	0.3 c.c.	Partial.
2	0.4 c.c. = 0.012 mg.	Partial.
3	0.8 c.c. = 0.024 mg.	Complete.
4	1.2 c.c.	Complete.

Cod liver oil is known to be composed of the glycerides of many fatty acids, some of which are much more unsaturated than oleic acid. A sample of the oil was saponified, the fatty acids separated, and their iodine value found to be 162. Had the only acid present been oleic this value would have been 90. This gives an idea of the extent to which acids more unsaturated than oleic acid were present in the mixture. A solution of the sodium soaps was prepared and used in the strengths indicated in table X.

TABLE XII.

Minimum Completely Hemolytic Dose in Milligrams for 0.5 c.c. of a 5 Per Cent. Suspension of Red Blood Cells of the Sheep.

Sodium soaps of	Iodine value.	Mg.
Oleic acid	87.5	0.03
Palmitic acid		> 0.04
Erucic acid	61.8	0.07
Linoleic acid	186.6	0.037
Dibromostearic acid		0.04
Chloriodostearic acid		0.04
Monobromostearic acid		0.03
α-brom[illegible]		0.03
Dihydroxystearic acid		2.4
Mixed acids of cod liver oil	162.0	0.024
Mixed acids of linseed oil	180.0	0.024

TABLE XIII.

Minimum Hemolytic Dose in Milligrams for 0.5 c.c. of a 5 Per Cent. Suspension [illegible] Corpuscles.

Case No.		Fraction 1 soluble in acetone.	Fatty acids from fraction 1.	Fraction 2 precipitated by acetone.	Fatty acids from fraction 2.
1. Cancer of prostate	Heart	>0.14	0.4*		
	Liver			>4.0	0.2
2. Cancer of prostate	Liver	0.04	0.022*	0.05	0.02
	Liver	0.4	0.14	0.35	0.6
3. Pneumonia	Mucosa of small intestine	0.0			
		0.21	0.18*		
4. Anemia not pernicious in type	Liver	>6.5	0.1	5.5	0.1
	Mucosa	0.11	0.11		
5. Pernicious anemia	Heart	0.6		2.0	
	[illegible]	3.0	0.37	2.7	
	[illegible]	>9.0	0.3*	2.5	0.1
			0.28		
	[illegible]	1.7	0.3*		
			0.17		
	[illegible]	1.3	0.45*	>3.4	0.07
			0.17		

Similarly linseed oil was used for the experiment to which table XI refers. The fatty acids obtained from this source had the iodine value 180, and were therefore still more largely composed of the acids more unsaturated than oleic acid than in the case of cod liver oil.

The results in tables X and XI show that the hemolytic power of these acids was somewhat stronger than that of sodium oleate, but on other occasions on which the experiments were repeated results were obtained in which the minimum hemolytic dose was larger than that shown in the tables, and no material difference therefore was demonstrable between the lytic properties of these acids and of sodium oleate, not sufficient at any rate to weaken the evidence given above for the statement that unsaturated fatty acids do not owe their activity as hemolytic agents to the chemical activity conferred on them by the unsaturated carbon atoms, whatever the true cause for their hemolytic properties may be.

We come now to the question whether toxic hemolysins of a fatty nature can be found in the body of patients dying of pernicious anemia, or in the subjects of other toxic forms of anemia.

Faust and Tallquist state that from 1 to 5 mg. of erucic, crotonic, or cinnamic acid causes intense or complete hemolysis of 10 c.c. of a 2 per cent. suspension of ox corpuscles in two hours at 37° C., and in intensity of action they are equal to oleic acid. They also state that the sodium salts of oleic and erucic acid are as strongly lytic as the free acids, while stearic and palmitic acids caused no hemolysis of red blood corpuscles of any species examined, when added up to any amount (*"beliebige Menge"*) as sodium salts. These statements are clearly not intended to be exactly quantitative. But if 10 c.c. of a 2 per cent. suspension is the same as 4 c.c. of a 5 per cent. suspension, then the dose of these substances as given by them is on the scale used in this paper, ⅛ to ⅝ mg., which is from four to twenty times too high in the case of oleic acid. A difficulty that has been generally felt in accepting the explanation given by these authors of the origin of this form of anemia is that oleic acid is constantly present in the body in considerable quantities. The blood, according to Hoppe Seyler, contains in the horse and dog up to 0.12 per cent. of soap, and in man, according to Kobert, as Meyerstein points out, the same amount. And it would seem that the protective agents existing in the serum, which Meyerstein and others have studied, cholesterol, neutral fats, and lipoids, ought to account for immunity from damage to the corpuscles even with considerable variations in the amount of oleate in the blood.

The other observers who have endeavored to ascribe the hemolysis in toxic anemias to fatty hemolysins have not confined themselves so precisely to one particular substance. Berger and Tsuchiya (7) prepared a 2 per cent. emulsion in normal saline of the ether extract of the mucous membrane of stomach and intestines from a normal human subject and also from a patient that died of pernicious anemia. In the former case five drops (of which forty-five went to 1 c.c.) were necessary to lake completely 5 c.c. of a 2 per cent. suspension of human corpuscles, or 2 c.c. of a 5 per cent. suspension: therefore 0.5 c.c. of a 5 per cent. suspension would require one fourth of this amount (¼ of $^5/_{45}$ of 20 mg.) or 0.55 mg. In the latter case, of the extract from the mucosa in pernicious anemia one fifth of this amount was sufficient; that is, 0.11 mg. for 0.5 c.c. of 5 per cent. suspension, or about four times as much as would have been necessary had the extract consisted of sodium oleate or oleic acid. Their figures show a noticeable difference between the hemolytic properties of the normal extract and the abnormal, but even in this latter case it would not appear that the potency of the material obtained in pernicious anemia was so great as to warrant far reaching conclusion[illegible].

Joannovics and Pick prepared from the liver in acute yellow atrophy in phosphorus poisoning in man, the dog, and the rabbit, and in dogs poisoned with toluylene diamine, an extract soluble in ether, in acetone, and in methyl alcohol, which they maintain was by means of these solvents freed from simple glycerides and also from complex fatty substances containing phosphorus, and was therefore substantially composed of fatty acids. Of this material the minimum completely hemolytic dose for 0.5 c.c. of a 5 per cent. suspension of dog's red blood corpuscles was as [illegible]

Acute yellow atrophy	0.013 mg.
Phosphorus poisoning (man)	0.062 mg.
Phosphorus poisoning (dog)	0.112 mg.
Phosphorus poisoning (dog)	0.065 mg.
Phosphorus poisoning (rabbit)	0.105 mg.
Phosphorus poisoning (rabbit)	0.088 mg.

The fatty acids found free in the liver in these five cases of phosphorus poisoning had therefore from a half to a quarter of the hemolytic power of sodium oleate or oleic acid, whereas in the case of acute yellow atrophy it was greater than this, about twice as great. Although, therefore, the effect of phosphorus did not bring to light any evidence for the belief that in the decomposition of the unknown fatty complexes of liver cells substances might be liberated with greater lytic properties than might well belong to the simple products of the hydrolysis of common fat, nevertheless the case of yellow atrophy bears out this conception. But if this is the explanation of the hemolysis in pernicious anemia, if pernicious anemia is a sort of chronic yellow atrophy, some explanation is required to account for the fact that the extensive destruction of liver tissue in phosphorus poisoning gives such different results. Joannovics and Pick draw different conclusions from their findings, both in these cases and in the case of extracts obtained from the liver in animals poisoned with toluylene diamine. In these latter experiments the data given do not allow of an exact comparison on these lines. The volume of the methyl alcoholic solution necessary for complete hemolysis is given, but not the amount of extract contained in that volume, but the authors themselves do not suggest that these results were more striking than those obtained in the cases of phosphorus poisoning, so we should not be justified in concluding that this was the case. More recently, however, Maidorn (8) has published results of experiments of this same nature, in which the method of preparing the extracts was the same as that employed by Joannovics and Pick, and he states that in his experiments the extract soluble in acetone from 100 gm. of liver, the weight of which is given, was made up to 100 c.c. of solution in methyl alcohol; a part, doubtless, of this extract did not dissolve in methyl alcohol, but we are not told how much did dissolve. Taking, however, the amount of extract soluble in acetone that it was necessary to use in order to get complete hemolysis in 0.5 c.c. (the author used 0.2 c.c.) of 5 per cent. sheep red blood corpuscles, we obtain the following figures:

	Extract in mg.
1. Normal liver (dog)	1.06
2. Normal liver autolyzed 48 hours (dog)	0.33
3. Acute toluylene diamine poisoning (dog)	1.60
4. Acute toluylene diamine poisoning after removal of spleen (dog)	3.80
5. Chronic toluylene diamine poisoning (dog)	1.50
6. Chronic toluylene diamine poisoning after removal of spleen (dog)	1.90
7. Acute phosphorus poisoning (dog)	2.30
8. Acute phosphorus poisoning after removal of spleen (dog)	4.80
9. Chronic phosphorus poisoning (dog)	7.40
10. Chronic phosphorus poisoning after removal of spleen (dog)	0.70

In each case we must suppose that only a certain fraction of the amounts dissolved in methyl alcohol; but for the hemolytic potency of that which did dissolve to be of the order of the hemolytic potency of oleic acid, we must suppose that this fraction was never larger than 1/10 (experiment 2) and ranged down to 1/250 (experiment 9). If as little of the acetone extract as this went into solution in methyl alcohol the author would surely have commented on the fact. It is difficult, therefore, to elicit from these results any evidence for the formation of hemolysins in these conditions, such as the author seems to have in mind; that is, so powerful as to introduce a new factor into the pathology of anemia.

In order to test the point with material obtained from the human subject in health and in pernicious anemia the following experiments were carried out.

Extracts were prepared as follows: The organ freed from all visible adipose connective tissue was minced, weighed, and treated with about 100 to 150 c.c. of alcohol for every 100 gm. of material. After twenty-four hours the aqueous alcoholic fluid was strained off on muslin, pressed out by hand, and discarded; the tissue was then shaken with the same volume of alcohol again; the alcohol pressed out in a screw-press the next day was kept, and similarly two or three further portions of alcoholic solution, obtained on the following days, were all mixed, filtered, evaporated *in vacuo* at about 40° C. to dryness, and the residue taken up in ether and filtered. After this extraction with alcohol the tissue, now practically free from water, was similarly extracted with ether, the ether similarly evaporated, and the residue taken up in ether and filtered. This ethereal solution was added to that obtained from the alcohol extraction and the mixture precipitated with acetone. The mixture of acetone and ether, filtered off and evaporated *in vacuo* at about 40° C., gave a residue which was taken up in acetone and filtered (fraction 1). The small amount which did not dissolve in acetone, added to that which had been precipitated by acetone, was dissolved in ether and precipitated again once or twice with acetone. In this way finally a second fraction was obtained insoluble in acetone (fraction 2). These two fractions were dissolved in methyl alcohol, neutralized to phenolphthalein with N/10 soda, the amount of dissolved material in a measured quantity of each solution was determined, and then the hemolytic power of each solution ascertained. A portion also of each fraction was saponified with alcoholic potash, made acid, and the fatty acids, thus set free, were shaken out with petroleum ether, and examined as to their hemolytic power after neutralization with soda. Solutions were obtained in this way from five cases.

Case 1. A man who died after operation for cancer of the prostate; there were no metastases and no cachexia

Case 2. Cancer of the prostate with metastases and some cachexia.

Case 3. Acute l[illegible]

Case 4. Anemia not typically pernicious, color index low, no signs of regeneration, no gastro-intestinal symptom

Case 5. A man who died of pernicious anemia, showing the typical blood picture of the aplastic type (1.2 to 1.6 million red corpuscles with high color index and no signs of regeneration, except a few nucleated red corpuscles) in this, his third attack of illness. He exhibited intestinal symptoms which were, however, not severe.

The first four cases were thus controls and should make clear the difference between the hemolytic properties of the fatty substances obtainable from the tissues in typical pernicious anemia (case 5) and those found in other conditions, if such a difference exists and if in pernicious anemia the disease is due to hemolysis by toxic fatty substances present in the tissues.

In table XIII the results obtained with material from these cases are summarized. Where the figures given are preceded by the sign >, the actual amount that caused complete hemolysis was not determined; the largest amount actually used did not have this effect and that figure is given. In every instance this is sufficient to prove that there was no unusually potent agent operating.

The figures in the second column (fatty acids from fraction 1) are in some cases marked with an asterisk. Fraction 1 would contain, in addition to simple glycerides and free fatty acids, cholesterol and its esters, if present. In most instances the portion of fraction 1 that had been saponified was shaken with light petroleum two or more times in alkaline solution to remove cholesterol and unsaponified matter; in some instances the products of saponification were tested both before and after this treatment, in others only after it.

The asterisks indicate tests where the treatment had not been carried out. The protective action of cholesterol which has been studied by Meyerstein and others is illustrated where the test was made both before and after the removal of unsaponifiable substances.

The examination of the figures as a whole shows that the hemolytic action of the substances soluble in acetone and of those precipitated by it is far smaller than that of the fatty acids that can be obtained from them; and further that the action of these fatty acids is generally not as strong as would be that of pure oleic acid by itself. And more especially the figures obtained in the case of the tissues in pernicious anemia fall entirely within the limits of those obtained similarly in other conditions and fail absolutely to give any indication of the presence of hemolytic toxins of the kind which have been supposed by some to be present and to be the cause of the disease.

It is true of course that the treatment to which the material was subjected before the fatty acids were finally isolated for the carrying out of these tests can hardly have been without effect upon the chemical constitution of some of the more sensitive ones. Although all evaporations were done in flasks from which air was removed by a fine stream of carbonic acid under low pressure, and at a low temperature, still the extraction of the tissues with alcohol and with ether necessarily lasted several days, and the action of air at the room temperature during this time within stoppered vessels was not excluded; and also, of course, the saponification with alcoholic potash[1] is known to be accompanied by changes in unsaturated acids. It may therefore be that the acids as used for the tests were in part different from those originally present in the substances contained in the cells. The changes known to be brought about by such operations, partial oxidation of unsaturated acids, and consequent lowering of their iodine value, would probably, as the experiments with dihydroxystearic acid given in the earlier part of this paper show, tend to diminish the hemolytic power of the acids finally obtained.

---

[1] In order to avoid heating with alcoholic potash, in one experiment the saponification by means of hepatic lipase was employed for the liberation of the fatty acids. Although this did not yield results other than those given in the table, this method has not yet been adequately tried out in the experiments here recorded and is to be submitted to further tests in this laboratory.

But the oxidized acids being insoluble in petroleum ether would be removed in the final operation for obtaining the fatty acids. And we find that at any rate in the case of pernicious anemia the fatty acids obtained have a lower hemolytic action than oleic acid, excepting only those obtained from the liver, which behave about as they would if they [illegible]

[illegible] IONS.

1. The smallest amount of the sodium soaps necessary for the complete hemolysis of 0.5 of a cubic centimeter of a 5 per cent. suspension of the red blood corpuscles of the sheep, ox, rabbit, dog, or of man, is about the same,—0.03 of a milligram in the case of the following acids: oleic, linoleic, dibromostearic, chloriodostearic, and two isomeric monobromostearic acids; in the case of erucic acid about twice as much of the soap was found to be necessary; in that of palmitic or of dihydroxystearic acid more than ten times as much.

2. The minimum hemolytic quantity of the sodium soaps of the highly unsaturated acids obtained from cod liver oil and from linseed oil is only very slightly less than that of sodium oleate.

3. It follows, therefore, from these results that hemolysis by unsaturated fatty acids is not more active in proportion to the degree to which these acids are unsaturated, nor is it diminished when the unsaturated carbon atoms are saturated by halogens. It is, on the other hand, greatly diminished when they are converted into the corresponding hydroxyl acids, which are hemolytic only to the same degree as th [illegible]

4. The idea that toxic hemolysis, in disease, in poisoning by phosphorus or toluylene diamine, results from the liberation of specially hemolytic fatty acids from the fatty complexes of disintegrating cells is not well supported by evidence; none of the fatty acids, still less any of the fatty complexes from which these acids can be obtained in any of the organs examined, either in this work or in the work of others that has preceded it, show on analysis any evidence for the existence of fatty acids more toxic than the common oleic acid which is constantly being set free by hydrolysis from common fat in health

BIBLIOGRAPHY.

1. Faust, E. S., and Tallquist, T. W., *Arch. f. exper. Path. u. Pharmakol.*, 1907, lvii, 367.
2. Joannovics, G., and Pick, E. P., *Ztschr. f. exper. Path. u. Therap.*, 1910, vii, 185; *Berl. klin. Wchnschr.*, 1910, xlvii, 928.
3. Noguchi, H., *Biochem. Ztschr.*, 1907, vi, 341.
4. Meyerstein, W., *Deutsch. Arch. f. klin. Med.*, 1912, cv, 79.
5. Lamar, R. V., *Jour. Exper. Med.*, 1911, xiii, 385.
6. Hartley, P., *Jour. Physiol.*, 1909, xxxviii, 353.
7. Berger, F., and Tsuchiya, I., *Deutsch. Arch. f. klin. Med.*, 1909, xcvi, 258.
8. Maidorn, R., *Biochem. Ztschr.*, 1912, xlv, 328.

Reprinted from the Transactions of the Tenth Annual Meeting of
The National Association for the Study and Prevention of Tuberculosis

BIOLOGICAL CURVES OBTAINED DURING THE ONSET AND COURSE OF TUBERCULOUS INFECTION

By Alfred H. Caulfeild, M.B., and F. S. Minns, M.B.
Toronto, Ont.

In 1911 one of us published preliminary reports* upon the effect of certain tuberculous sera upon tuberculo-antigen. This effect was estimated by the lessened adsorption that antigen-serum mixtures had for complement in contrast to that of the corresponding antigen-salt mixtures. This phenomenon is exactly the reverse of complement fixation, and although the technic is an adaptation of the latter, the protocol necessary for the routine demonstration of this reverse phenomenon differs from the necessary arrangement for complement fixation.

As the purpose of this paper is to demonstrate the relation between the clinical progress and biological curves obtained by the repetition of these test-tube reactions, together with repeated quantitative estimations of the tuberculin sensitiveness, we do not propose to deal extensively with the technic of the methods, which have been fairly fully outlined in the former publications. The technic therein given is essentially the basis of the present report, although various modifications have been adopted to increase the delicacy and accuracy of the test. One important detail of the technic might, however, be mentioned, and that is the necessity of estimating the natural hemolytic strength of the serum to the corpuscles of whatever system used. In the former reports this factor was controlled rather than eliminated. Subsequently we found it consumed less time and gave greater accuracy to first absorb the natural hemolysin by incubation of the inactivated sera with washed sheep-corpuscles.

As we have found it necessary, for convenience of expression, to introduce two terms descriptive of the end-results of the test-tube reactions, a brief discussion of the nomenclature at this point may facilitate the subsequent use of these terms.

The technic adopted to demonstrate this reverse phenomenon to complement fixation is based upon the fact that all antigens are capable of non-specifically adsorbing complement in various degrees. Now, if to an appropriate series of antigen dilutions, whose complement-adsorbing capacities are quantitatively shown, sera are added, it will be found that certain sera give—(1) true complement fixation (the demonstration of this taking place in appropriate antigen dilutions where only minimal parts of a unit of complement are adsorbed; (2) others will show no effect (indifferent reaction); (3) others will so react with the antigen that these antigen-serum mixtures show great loss of complement-adsorbing power (inhibitive reaction). Experiments have been given regarding the specificity of this latter, and will be enlarged upon in a later publication.

---

* Caulfeild: "Investigations on Pulmonary Tuberculosis," Jour. Med. Research, January, 1911, p. 128; Caulfeild: "Correlation of Clinical Progress with The Results of Immunological Studies," Arch. Int. Medicine, October, 1911, vol. viii, p. 440.

Taking the phenomena in the reverse order, the following remarks may help to explain the method adopted to record graphically the end-results:

1. *Inhibitive Phenomenon.*—These sera so digest or react with the antigen* that, in contrast to the antigen-salt mixtures, the corresponding antigen-serum mixtures show a loss of capacity for complement adsorption. Different sera show this phenomenon in different degrees, and the protocol experiment is arranged to demonstrate this quantitatively. For convenience of expression the phenomenon has been termed the inhibitive reaction, in contrast to complement fixation. In reality it is the inhibiting effect on what was formerly, before the addition of serum, the power of that amount of antigen in the same degree of dilution to non-specifically adsorb complement—estimated quantitatively. The protocols are arranged so as to show this power in three degrees thus: Where the amount of antigen-adsorbing power equals four units of complement the inhibitive reaction would be called complete if the corresponding antigen-serum mixture showed an adsorption of not more than one unit of complement, *i. e.*, complete hemolysis, and the potentiality of releasing three units. In the nomenclature adopted in the curves this is $I._1$; if the antigen-serum mixture showed an adsorption potentiality of two units, $I._2$, and of three units, $I._3$.

2. *The Indifferent Reaction.*—These sera react in no way with the antigen, that is, if the capacity of each serum to non-specifically adsorb complement be estimated and considered in the results obtained in the antigen-serum mixtures it will be seen that the effect upon complement is the same as in the corresponding antigen-salt mixtures. In other words, neither the reaction of fixation nor the inhibitive reaction is given.

3. *True Fixation of Complement.*—Only those sera which show no hemolysis in the tubes where the antigen dilutions are capable of adsorbing only a minimal part of a unit of complement are accepted as positive. In the plotted curves what would be ordinarily called a + + + reaction is termed Fixation 1, to correspond with the terminology used for the inhibitive phenomenon.

The estimation of the tuberculin sensitiveness (or hypersensitiveness) has been made by the von Pirquet cutaneous method, with the technic described in a former publication,† except latterly, when for a time both the cutaneous and the intracutaneous method of Mantoux have been used. The amount of tuberculin has been, with the former, 10 c.mm. of a 50 per cent. solution of O. T., and the degree of sensitiveness judged from the extent of the reaction. In selected cases it has been possible to take readings every day. This method has given us more satisfaction than a determination of the minimal reacting dose, inasmuch as it seems as accurate, gives a greater amplitude in the results, and requires only one application in contrast to the two or more necessary to gauge exactly the minimal dose which will give a reaction. For the same reason we are now using a $\frac{1}{20}$ mg. O. T. in 20 c.mm. for the intradermal test, and gauging the degree of sensitiveness by the extent of the reaction.

---

* Attention should be drawn to the fact that this particular antigen is an alcohol-ether extract of tubercle bacilli, and that true complement fixation is obtained with it in only a very small percentage of cases. This low percentage is in very marked contrast to that obtained with most other tuberculo-antigens. These percentages are outlined in Table 1. (See p. 3.)

† Jour. Med. Research, vol. xxiv, No. 1, p. 103, 1911.

The earlier reports were based upon part of the investigation carried out on the sanatorium cases shown in Table 1. Owing to the increased scope afforded by the Tuberculosis Clinic of the Toronto General Hospital, the work has again been taken up, and now represents observations upon a much greater number than that outlined in Table 1, which was compiled at a time when it was convenient finally to sum up the cases investigated up to that particular date. We feel that the additional observations would make no material change in the percentage results.

TABLE 1

Number of Cases	Where Obtained	Number of Tests with Alcohol-ether Extract	Number of Tests with Other Tuberculo-antigens
154	In sanatorium..........	322	42
204	In Out-door Department, General Hospital.......	282	34
	Total..............	604	76

Of this number, 101 cases had had an average of 3.4 tests with alcohol-ether antigen.

True complement fixation was obtained in:	Sanatorium cases in:	34 per cent. cases with other antigens.
True complement fixation was obtained in:	Outdoor Department, General Hospital:	35 per cent. cases with other antigens.
True complement fixation was obtained in:	Sanatorium cases:	7.6 per cent. cases with alcohol-ether antigen.
True complement fixation was obtained in:	Outdoor Department:	4.8 per cent. cases with alcohol-ether antigen.
Full inhibitive reaction:	Sanatorium cases:	22.4 per cent. cases with alcohol-ether antigen.
Full inhibitive reaction:	Outdoor Department:	29.4 per cent. cases with alcohol-ether antigen.

The above results are compiled from data on cases which were investigated while they were resident in sanatoria, and upon whom in general no further observation has been possible. The remaining cases are those investigated in a routine fashion at the Tuberculosis Clinic of the Toronto General Hospital up to March 15, 1914, as this date was a convenient one to take for a review of the cases from both a clinical and a biological point of view. The work, however, has been carried on from that time, and explains in a few of the charts additional data obtained after March 15th.

As well as the observations upon human beings, considerable work has been done with cows and rabbits experimentally injected with different strains of acid-fast bacilli. In only one instance out of about 40 rabbits has the inhibitive reaction been obtained. This occurred in one rabbit which successfully withstood the inoculation of 1,400,000,000 of living bacilli. This strain was obtained from an abscess following a Friedmann inoculation. True fixation can be obtained in rabbits* with the alcohol-ether antigen as well as with other tuberculo-antigens. In contrast to the results in small laboratory animals, the reaction has been observed in all degrees in cows. Work is at present under way with these animals to correlate these findings with other data.

As it would be impossible in this report to deal with the material that has accumulated in these investigations by analyzing each case, although it is by this method, as well as by subsequent groupings, that we have come to the conclusions we now hold, our purpose will best be fulfilled, it would seem, by presenting the results under various groupings, and by further illustrating the various points by charting the results obtained in particular cases.

A. Results Obtained in Clinical Normals.—In the beginning of these investigations samples of blood from clinical normals were used for the purpose of "controls." However, as the application of the tests developed, and as variations both in the test-tube results and the clinical life of certain of the controls were obtained, the conception was formed that from just such material data might be obtained which would throw light upon "immunity to tuberculosis." No doubt this hope originated in the deductions drawn from the results of tuberculin tests on healthy subjects and postmortem statistics regarding the prevalence of the lesions of healed tuberculosis. These allow one to believe that during the life of different individuals exposures to tubercle bacilli and bacillary implantations take place, and that, in those remaining clinically free from tuberculosis, the implantations are successfully resisted within varying periods of time and varying degrees of biological reaction and anatomical involvement. Our belief is that we obtain immunity to tuberculosis (by which is meant definitely established clinical tuberculosis) through exposures or implantations less in amount or virulence than our resistance, and that our resistance or immunity is fostered by these successfully resisted implantations. Whether we regard reinfection to be caused from within or without is not material to the point at issue.

Thus it has seemed to us that if these biological results can be of value (as they seem to be) in the definitely tuberculous, they should, if carried out continuously upon a sufficient number of clinical normals, throw some light upon the problem of immunity. In this way only would it seem possible in humans to contrast the reactions existent before, during, and after successfully resisted implantation. The contrast to this in the reactions following various degrees of early clinical infection is, of course, easily obtained. The value one will attach to the results in this connection will depend upon each observer's belief in the possibility of the tests representing a biological state (or physical condition depending on a biological state) and their specificity.

The list as it at present stands in the class of clinical normals is perhaps far from ideal, but a number of difficulties have arisen that were unforeseen in the beginning. The work was interrupted for about a year, and again observations were begun on individuals, who for various reasons were not again available for further tests. As would be expected, the most consistent series of observations has been obtained upon physicians, their friends, and medical students.

Table 2 gives the total number of cases included in this list, grouped under the year of the initial observation and the biological classification. It is noticeable that with increase of the inhibitive phenomenon there is roughly an increase in the tuberculin sensitiveness. Four cases have shown variations which we consider outside the error of the technic. Of these, No. 4 developed clinical tuberculosis. The lesion was a slight one of the right apex, and appeared to be an activation of a former infection. The diagnosis was agreed to by an independent worker in tuberculosis, who had no knowledge of the laboratory findings. This case, a laboratory "Diener," made excellent progress without entirely giving up his work at any time. He was alive and well and performing manual labor in 1913. Case No. 17 is the only one of the four showing variations whose findings are not charted in Fig. 1. This was not done because sufficient time had not elapsed for more than two closely spaced blood examinations. Case No. 14, the only instance of a full inhibitive phenomenon not subsequently giving a variation, is a physician who is spending most of his time in tuberculosis work; his second blood test was between $I._1$ and $I._2$.

---

* Caulfeild: Proc. Royal Society, B. vol. lxxxiv, p. 390, 1911.

TABLE 2

YEAR OF FIRST OBSERVATION	NO. OF CASE	RESULT OF FIRST TEST-TUBE REACTION				TUBERCULIN SENSITIVENESS
		$I._1$	$I._2$	$I._3$	IND.	
1910–11........	1	..	..	..	+	16 x 16 mm.
1910–11........	2	+(V.)	..	..	..	Variations marked.
1910–11........	3	..	+	..	..	Slight variations.
1910–11........	4	..	..	..	+(V.)	25 x 30.
1913..........	5	..	..	+	..	12 x 12.
1913..........	6	..	..	..	+	Negative.
1913..........	7	..	..	..	+	Negative.
1913..........	8	..	..	..	+	10 x 12.
1913..........	9	..	..	+	..	15 x 18.
1913..........	10	..	..	..	+	Negative.
1913..........	11	..	..	..	+	Negative. (?)
1913..........	12	..	+(V.)	..	..	Variations.
1913..........	13	..	..	+	..	12 x 12. (?)
1913..........	14	+	..	..	..	25 x 25.
1913..........	15	..	..	..	+	10 x 15.
1913..........	16	..	..	..	+	15 x 15.
1914..........	17	..	+(V.)	..	..	10 x 12. (25 x 40)
1914..........	18	..	..	..	+	Negative.
Totals...	..	2	3	3	10	

Up to the present the total number of observations made on the above is 53.

The letter V. means that subsequent tests showed a variation of more than one degree of the nomenclature (a variation of one degree only being considered within the range of experimental error). Four cases, including the one case of positive clinical tuberculosis, have shown variations, *i. e.*, 22 per cent. Case No. 2 developed tuberculosis during the observations. Case No. 17, a research worker, has shown slight variations, gave complement fixation with a bacillus emulsion antigen, and a general febrile reaction of 101° with 0.2 mg. O. T. given subcutaneously.

In Fig. 1 an example is given (Case No. 1) of repeatedly similar results. Case No. 3, a physician working in tuberculosis, has only had two blood tests, but as far as they are concerned the variation is considered as easily due to the possible error of the technic or interpretation. The rough correlation that seems to exist between a high inhibitive reaction and marked tuberculin sensitiveness is well shown. Case No. 2, already previously reported in 1911, was suspected of clinical tuberculosis, although the actual clinical demonstration was lacking. In this case a very marked change in the character of the cutaneous reaction was noticeable between the second and third tests. The maximum intensity was reached on the fourth day in the second test, and on the beginning of the second day in the third test; the fourth test was also more rapid in its onset than the second.

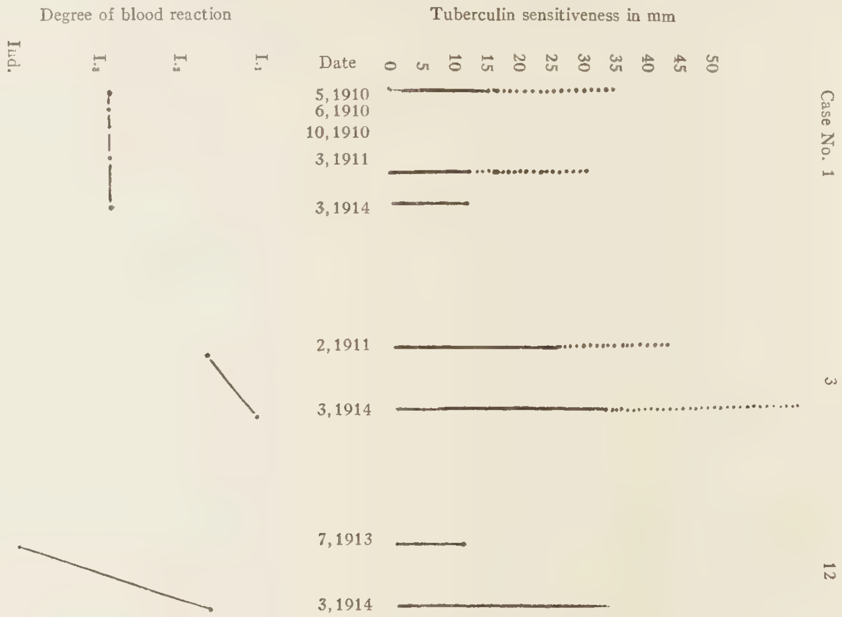


Fig. 1

The date shows only the month and the year: the spacing is only approximate to avoid unwieldiness.

The dotted lines of the tuberculin reaction signify that amount of halo around the infiltration.

The significance of the rapid onset of the day of maximum intensity of this reaction is referred to in the discussion of these cases.

Case No. 12 remarked before the second blood test that he had been slightly troubled with his knee; a clinical examination revealed no evidence of any

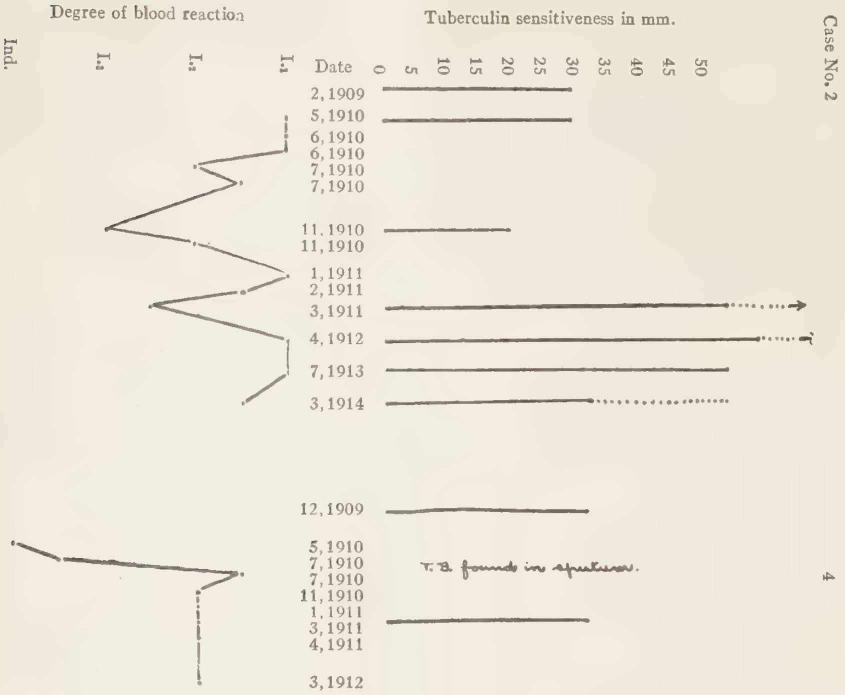


FIG. 1 (*Continued*)

character, however. The tuberculin test was performed a week or so later. It should be remarked here that there is no possible chance of a bias on the part of the one who reads the end-results. All samples of blood are inactivated, have their hemolysin extracted, and are put up under a number.

The reason why the cases giving variations have had relatively more observations made on them is because once a variation is obtained much more endeavor is made to get a repetition. Further, as this represents work continued over a long period of time, it has very frequently been impossible to get these cases when desired.

B. Results Obtained in Cases of Definite Exposure.—This class is very small in number, because we have included in it only cases for whom the exposure has been very definite, and where there was no evidence whatever of a clinical manifestation of tuberculosis. It does not include the cases which in a routine manner are collected in a tuberculosis clinic when any one in the household is found to have contracted tuberculosis. Sufficient time has not elapsed to allow us to check this material, for whom the exposure generally is still continuing. Further, no case fulfilling the conditions outlined has been included until a sufficient period of time has successfully elapsed, so that one is as certain as possible that clinical tuberculosis has not resulted.

Table 3 shows the blood reactions of these few cases, together with the amount of the tuberculin sensitiveness. Three cases coming in the $I._1$ column show practically negative results. In Case 2 this is easily explained by the duration since the exposure. In Cases 5 and 7 this cannot be the explanation. We feel that it is, however, probably due to the long duration of the exposure. This was the explanation offered by one of us* in 1911 for the rarely found negative tuberculin tests obtained in undoubted cases with a slight involvement and good prognosis.

TABLE 3

No. of Case	$I._1$	$I._2$	$I._3$	Notes regarding the duration of the exposures, and time since exposure that blood was examined.
1	20 x 30	..	..	Exposed for about a year; about six months after the exposure.
2	5 x 5	..	..	Exposed for about a year; fourteen months after the exposure.
3	..	30 x 30	..	Exposed on and off for two and a half years: about three months after exposure.
4	15 x 15 25 x 30	..	..	(See note below)
5	5 x 5	..	..	Exposed for many years; one month after exposure.
6	..	30 x 40	..	Exposed for about six months; six months after exposure.
7	10 x 10	..	..	Exposed for many years; six months after exposure.

The numbers represent the millimeter measurements of the cutaneous test. Case 4 gave a remarkably clear-cut result, aged twenty-two, had been intimately exposed to one of the opposite sex; previously not likely to have had any exposure. About one month afterward patient sought medical advice, not because of any complaint, but because he only then learnt that he had been exposed to tuberculosis. The cutaneous test at that time (June, 1913) measured 15 x 15 mm.; in August a full inhibitive reaction was obtained, and in November, 1913, the cutaneous test measured 25 x 30 mm.

While we feel that seven cases are not sufficient from which to draw conclusions, still the contrast between the high degree of the inhibitive phenomenon found in all the exposed cases is in marked contrast to that obtained in the clinical normal group. This is even more marked if one excludes from the clinical normal group those who showed no variation in the blood or tuberculin tests, or who were exposed to tuberculosis, as were the physicians working extensively with that disease.

---

* Caulfeild and Beatty: Jour. of Med. Research, 1911, vol. xxiv, No. 1, pp. 110 and 116.

Thus if one excludes from the clinical normal group those whose blood and tuberculin tests showed variations, those physicians whose work brought them in constant contact with cases, and the one case in which tuberculosis developed, it leaves all the remaining examples in the I_3 or indifferent class. Also it leaves all the undoubtedly exposed cases and those doubtful clinical normals at one time or another in the two higher grades of the inhibitive phenomenon. In other words, the two tests make it possible to take from the clinical normal class certain ones which give the same findings as the undoubtedly exposed cases, which still are free from clinical tuberculosis. That is, these clinical normals were exposed and received their tubercle bacilli implantations.

C. Results Obtained in Cases Giving Doubtful Evidence of Pulmonary Tuberculosis.—It is perhaps as well here to make some observations regarding the clinical classifications adopted, under which the results of the biological findings are shown. It is always a difficult thing—if not impossible—to divide patients into tuberculous and non-tuberculous groups, and this is especially true in a busy tuberculosis clinic of a general hospital. On the staff of such a clinic there naturally must be differences in skill and expertness in technic and interpretation. In view of these conditions it was felt by us that it would perhaps be easier and less influenced by subjective error to form this list (Table 4) by excluding those cases, whom the one or two physicians who examined the patients felt without doubt were cases of tuberculosis; and by further excluding those cases which had already been discharged from the clinic as non-tuberculous. These restrictions probably resulted in the inclusion of a greater percentage of the tuberculosis free than would otherwise have resulted had further subdivision been adopted. It is often easier to make a diagnosis than to exclude the possibility. The only laboratory result playing a part in the classification was the sputum report. These cases then might be summed up by the statement that they included those in whom it was possible that other pathologic conditions might explain the symptoms and findings, or were those whom it seemed advisable to keep under observation because tuberculosis at the moment could not be excluded.

On these cases, the tests have usually been of value only when their repetition showed variations, and the variation, if sufficiently marked, corresponded with the clinical course. This involves naturally a very careful consideration and review of each case. Now, as deductions drawn by any particular interpreter must to a certain extent be biased, the proof of the correctness of the basis of the interpretation must rest upon a large number of cases. It would be impossible in this communication to detail a sufficient number of cases to meet this just criticism, nor do we feel that at present sufficient material has been worked over. Such cases as these in tuberculosis often require many months to furnish the proof of the correctness of the diagnosis. No more than one or two examples will be given to indicate the basis of the interpretation.

The following cases illustrate the aid afforded by the reactions. They are all, as already outlined, cases in whom the diagnosis could not positively be made, nor could tuberculosis be excluded. The classification, of course, is based upon a consideration of both the physical findings and the history.

(1) Hospital No. 1433. The blood reaction on December 11, 1913, was I.$_3$, at which time the cutaneous test measured 15 x 15 mm. On April 1, 1914, both reactions were the same, but in the meantime the patient had lost the suggestive symptoms. Our conclusion was that some other pathologic condition explained the clinical complex found at the time the patient first presented himself.

(2) Hospital No. 782. The blood reaction on December 20, 1913, was I.$_2$, and the cutaneous test measured 30 x 30 mm. On April 1, 1914, the blood reaction was found to be the same, and although the cutaneous test was overlooked, the clinical improvement enabled us to support the diagnosis in the same manner as in the preceding case.

(3) Hospital No. 4124. Between August, 1913, and March, 1914, four blood tests were made. All varied between I.$_3$ and I.$_2$. Slight variations were obtained in the cutaneous tests. The clinical picture remained stationary or slightly worse. The diagnosis was made of positive tuberculosis. Incidentally, in support of this, a positive complement fixation with other tuberculo-antigens and a negative Wassermann were obtained.

(4) Hospital No. 4122. Three blood tests were made between August, 1913, and May, 1914, and two cutaneous tests. The blood results were as follows: Ind. I.$_3$, I.$_2$. The cutaneous tests were 5 x 5 mm. and 25 x 30 mm. Marked clinical improvement took place during this period. This strengthened the opinion that the patient had been suffering from tuberculosis.

(5) From August, 1913, to March, 1914, three blood tests were made, with the following results: Fixation, Ind. and I.$_2$. Clinically increased well-being helped a positive diagnosis. In this case only one cutaneous test was performed. The clinical picture included enlarged abdominal glands (?), and needed an exclusion of new-growths.

Of these five examples, it can be said that the first two cases gave results which might be duplicated among the clinical normals (see Table 2 and Fig. 1); that Case 4 is very similar to the exposed Case No. 4 in Table 3 and Fig. 1, and that the results, both biological and clinical, in Cases 3 and 5, are frequently encountered among the definitely tuberculous (Fig. 2).

It so happens that one of us has comparable data (Table 4) obtained on 30 cases resident in sanatoria. In but few of these did the writer take any part in the clinical classification. Many of the cases had been resident in the sanatoria for long periods of time, and had experienced the benefit of the change from their former life to the better conditions existing in the sanatoria. Most of the patients were at a free sanatorium, and as such represent a weeding-out of the cases of tuberculosis clinics in the cities. Further, if such cases were regarded as doubtful after a period of time in a sanatorium, they were likely to be those who had the power to improve fairly rapidly when given a better chance than that afforded by former circumstances. There is, of course, the possibility that some of them were tuberculosis free and got their exposure in the sanatorium, thus explaining the biological reactions. The contrast between the two sets of results is marked.

TABLE 4

DATA OBTAINED FROM TUBERCULOSIS CLINIC IN GENERAL HOSPITAL

No. of Case	I_1	I_2	I_3	Ind.	Fixation
1	..	18.22	..	..	..
r 2	20.25	..	..	..	..
3	..	Neg.	..	..	..
4	..	..	10.10	..	..
5	10.18	..	..	..	..
6	..	..	Neg.	..	..
7	10.	10	..	..	..
8	..	18.	18	..	..
r 9	25.30	..	..	..	..
10	Neg.	..	..	..	..
11	..	10.	10	..	..
r 12	..	..	..	–	–
r 13	15.35	..	..	..	..
14	10	10	..	..	..
r 15	..	..	10.	10	..
r 16	..	..	8.8	..	..
17	..	..	..	–	–
18	—	..	..	..	..
19	..	12.15	..	..	..
r 20	..	5.10	..	..	..
21	..	—	..	..	..
22	..	..	10.	15	..
23	..	..	20.20	..	..
24	..	Neg.	..	..	..
25	..	12.15	..	..	..
26	..	10.10	..	..	..
27	..	..	..	25.25	..
28	..	..	..	5.5	..
29	..	—	..	..	..
30	..	..	—	..	..
r 31	10.10	..	..	..	..
r 32	12.15	..	..	..	..
33	18.50	..	..	..	..
r 34	..	..	..	..	..
35	–	–	..	..	..
r 36	–	–	..	..	..
r 37	..	20.22	..	..	..
r 38	5.5	..	..	..	..
39	..	30.30	..	..	..
r 40	5.5	..	..	..	..
41	8.8	..	..	..	..
42	..	..	—	..	..
r 43	5.5	..	..	..	..
r 44	..	15.15	..	..	..
Respective totals.	13 4	12 2	6 2	2 2	

DATA OBTAINED ON CASES IN SANATORIA

No. of Case	I_1	I_2	I_3	Ind.
1	..	—	..	..
2	..	..	8.	10
r 3	25.30 (50.50)	..	..	..
4	..	..	—	..
5	30.40	..	..	..
6	..	..	..	12.15
7	..	..	15.15	..
8	30.30 (50.50)	..	..	..
9	18.18	..	..	..
10	35.35	..	..	..
11	22.25	..	..	..
12	—	..	..	..
13	..	Neg.	..	..
14	..	18	20	..
15	20.20	..	..	..
r 16	15.18	..	..	..
17	40.40	..	..	..
18	..	10.12	..	..
19	..	..	..	15.18
20	40.60	..	..	..
21	20.25	..	..	..
r 22	25.30	..	..	..
r 23	30.35	..	..	..
r 24	20.25	..	..	..
r 25	20.20 (40.40)	..	..	..
26	..	..	25.28	..
r 27	..	..	..	5.5
28	..	..	..	10.12
r 29	10.12	..	..	..
30	—	..	..	..
Respective totals...	18	3 1	3 1	4

The numbers underneath the biological columns, which indicate the results of the blood test, are the measurements in mm. of the tuberculin cutaneous test. A distinct halo to the cutaneous test is indicated by the numbers in parentheses.

When the blood test could not definitely be put in either of two classifications, the measurements of the tuberculin reaction are put in both columns to indicate this.

The letter r opposite the case number means that repeated observations have been made.

D. Results Obtained Upon Cases of Definite Pulmonary Tuberculosis.—It has been difficult to choose, from the various biological curves which have been obtained in this class, the few which would best illustrate the wide variety, their clinical correlation, and at the same time show the restrictions which must be remembered in a combined clinical and biological interpretation.

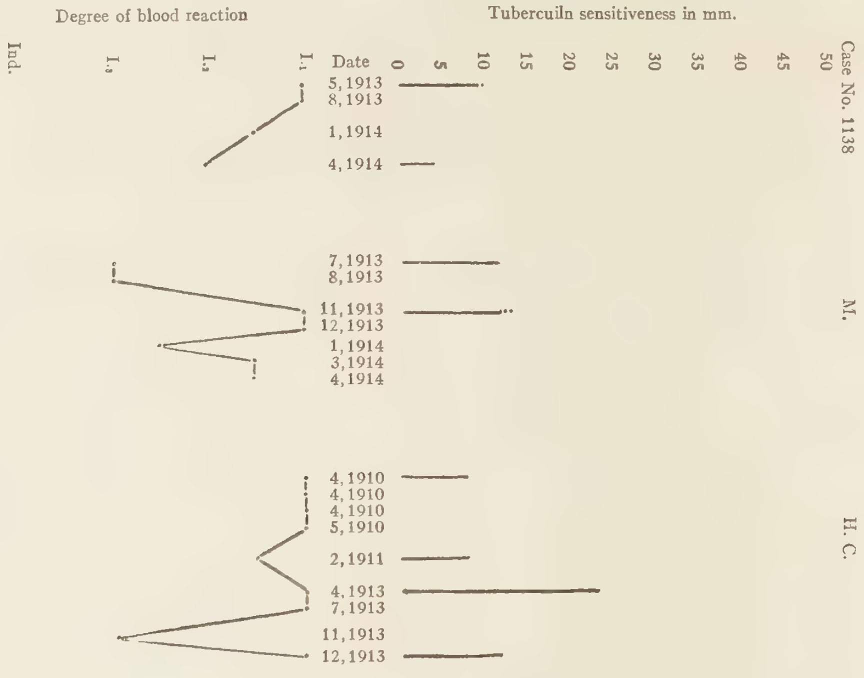


FIG. 2

In Case 1138 the initial observation was made during pregnancy; as previously the patient had been rapidly retrogressing, a Cæsarean section was under consideration. On account of the biological finding no interference was made and the case went to labor in November, 1913, with marked improvement. The slight drop subsequently may be the error of technic. The patient has remained in the better condition. Case M., a far-advanced involvement of both lungs, was running high fever at the time of the initial observations. Clinical improvement after the second observation was marked, locally and symptomatically. About the time of the fourth observation acute tuberculous laryngitis set in, which is apparently slowly improving. Case H. C., after returning from a sanatorium to work had an outbreak while not under observation in 1912. Since then he has done a full day's work in an office, with the one exception when he had a slight outbreak beginning about November, 1913, at which time tubercle bacilli were again obtained in the sputum. Is well and working at the present time. The change in the tuberculin sensitiveness before the outbreak is interesting.

The principal facts seem to be as follows:

(*a*) Increase of the inhibitive reactions precedes or accompanies favorable clinical progress; this is more marked the earlier the lesion, and seems more definite the more marked the tuberculin sensitiveness (or in a few cases the greater the tuberculin tolerance). Several instances of this are given in Fig. 2.

(*b*) Marked fixation, when sustained, is found in an entirely different type of case, which is nearly always febrile (Fig. 3).

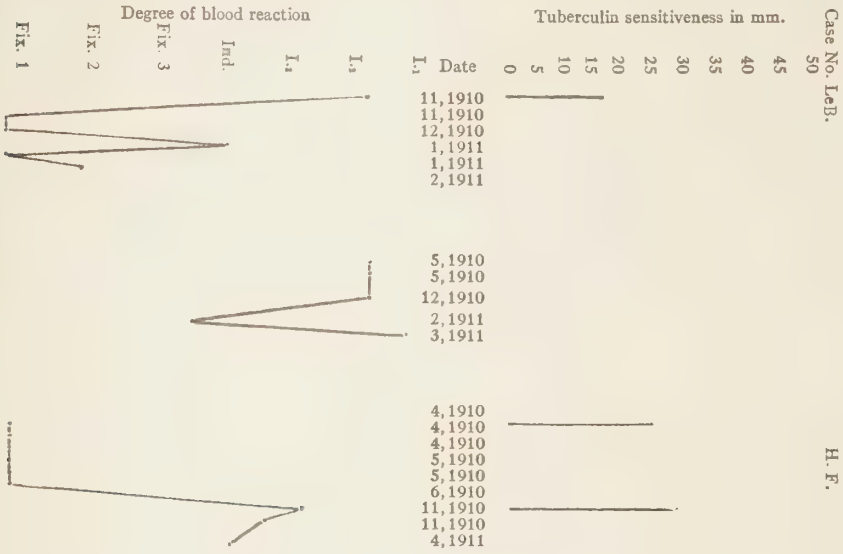


Fig. 3

These three cases represented in the beginning different amounts of anatomical involvement. All showed marked and rapid outbreaks of fever and retrogression from periods of comparative well-being. These outbreaks seemed to correspond with the blood results as accurately as the frequency of the tests allowed one to judge. Case H. F. J., acutely ill with temperature during part of 1910, improved considerably at the time the blood gave slight inhibitive reactions. Later, after a very marked retrogression during the summer of 1911, at which time the prognosis was apparently hopeless, a most dramatic recovery was made both focally and symptomatically. Unfortunately, tests could not be made at this time, so that it is only conjecture that he would have given a full inhibitive reaction, but consistent with daily findings with such a clinical picture.

(*c*) Sudden loss of inhibitive reaction or the development of fixation seems always to be accompanied by a clinical outbreak.

It is difficult to present percentage results regarding the correlation of these biological curves (specific to the tuberculous infection) with clinical variations, for such reasons as the difficulties of absolute diagnosis, the possible rôle of secondary infections, or any complication which makes the estimation of what is due solely to the tubercle bacillus an opinion rather than a fact. In certain far-advanced cases of ultimately hopeless prognosis we have obtained various degrees of the inhibitive phenomenon. Many of these cases have survived months longer than was at first deemed likely, or were apparently carried off by some complication. In none of these cases has the tuberculin sensitiveness been marked. However, nearly, if not all, the cases whose blood has been examined a short time before death have given the indifferent reaction.

E. Results Upon Other Forms of Tuberculous Infection.—Beyond a number of cases of gland involvement, few observations have been made on the remaining types of tuberculous infection, sinus, bone, etc. Only one of the cases of tuberculous glands has given a complement fixation result; all the others have shown various degrees of the inhibitive phenomenon. There has been only one case of supposed tuberculosis of the peritoneum. This case was pronounced undoubtedly one of tuberculosis, even at the time of operation. From the degree of tuberculin sensitiveness and the extent of the inhibitive phenomenon, in consideration with the acuteness of the case, we did not consider that tuberculosis could be the cause of the clinical picture. Inoculation of a guinea-pig with the peritoneal exudate failed to cause tuberculosis. Shortly after the operation the autopsy revealed extensive carcinoma. A circular fibrosis in the intestinal wall, noticed at the time of the operation, was the chief cause for the surgical diagnosis; the postmortem revealed the lesions of healed tuberculosis.

SUMMARY

The facts as presented seem to give strong support to the following statements:

1. An initial tuberculous implantation, where successfully resisted, produces a marked ability of the serum to react with an alcohol-ether extract of tubercle bacilli, as is shown by loss of the power of the antigen-serum mixture to adsorb complement, in contrast to this ability of a comparable antigen-salt mixture.

2. A reactivation or a reimplantation, when successfully resisted, results in an increase of the ability of the serum to react with antigen, but may not in all cases increase the tuberculin sensitiveness, which indeed in a few cases seems to be so slight that a negative von Pirquet skin test results with 10 c.c. of 50 per cent. old tuberculin.

3. Whatever phenomenon the reaction may represent, its estimation under certain restrictions is of practical value for certain cases in diagnosis, for others in prognosis, and in both it becomes an aid in treatment.

Reprinted from the Transactions of the Tenth Annual Meeting of
The National Association for the Study and Prevention of Tuberculosis

TUBERCULIN TREATMENT BASED UPON CLINICAL AND BIOLOGICAL DATA

By Alfred H. Caulfeild, M.B., and F. S. Minns, M.B.
Toronto, Ont.

The method is based upon the data presented in the preceding paper, and the administration is made in a manner to encourage the different biological combinations, which have been found to correspond, in the non-treated cases, with clinical improvement.

The principles underlying the selection of cases and the method of administration might be gathered together as follows:

(*a*) To improving (or stationary) cases, where the blood test shows a full inhibitive reaction, tuberculin is given in extremely small doses, with little or no increase, at about seven-day intervals, in all cases showing marked tuberculin sensitiveness; to all cases showing a tendency to tuberculin tolerance it is given in increasing amounts twice weekly. Occasionally this selection will include cases with fever. The tentative theory is that tuberculin sensitiveness may be maintained or increased, which, together with the inhibitive reaction, is one favorable biological combination, and by the second method tolerance may be aided, which with the inhibitive reaction is also a favorable biological condition.

(*b*) Cases whose blood shows complement fixation (to the alcohol-ether antigen) are never given tuberculin while this phenomenon remains.

(*c*) Certain cases whose blood test gives an indifferent result or merely a trace of the inhibitive phenomenon, present insufficient data for an exact selection of either method. Either method may be used according to one's judgment of which combination would likely develop under improvement. Further, as many of these biological results may be found with different clinical pictures, it is often not only a question of what method, but also whether tuberculin should be given. In our opinion usually it should not.

(*d*) It has been found by practical experience that those showing the second combination under heading (*a*) stand with benefit slight general reactions. For the second method we have used Koch's old tuberculin for the first bacillen emulsion.

(*e*) It will frequently be found that cases giving the combinations under heading (*a*) do not do as well as might be expected. In these cases we think we have a direct indication for autogenous vaccines.

Although this method of tuberculin administration (and vaccine therapy) has been used by us with a great number of cases for several years, there seems to be no way in which a statistical study can justly be made. That is, we have on untreated cases no comparable data, in which other circumstances incident to the welfare of the patient were sufficiently under control to give the statistical result a real value. Indeed, such a study of the results of any treatment for tuberculosis is almost necessarily confined to sanatoria, in which the full control of all the circumstances must be very ideal, and the numbers of each group large. Further, it does not seem logical to us to draw conclusions from results obtained after the patients have left the strict conditions necessary for proper comparison. To draw up statistical results based upon the condition of patients after they have passed from full control introduces an unknown factor large enough to offset the value of the comparison.

In fact, publications either for or against tuberculin administration base their conclusions, especially if extreme, on an opinion for or against rather than upon any convincing statistics. Under our circumstances we prefer to state that we favor tuberculin in selected cases. Further, we feel that the above method is based upon both laboratory data and clinical judgment, that it gives a means of excluding cases, of selecting cases for either of two methods, and still allows considerable individualism.

This method of selecting the case and the manner in which the tuberculin is to be administered is in use at the Tuberculosis Clinic at the Toronto General Hospital for all cases, irrespective of the type or location of the lesion. It is hoped in this way to determine if it can practically be applied where often different physicians have to continue the treatment.

THE EXCRETION OF NITROGEN IN FEVER

[Reprinted from the JOURNAL OF EXPERIMENTAL MEDICINE, Vol. XX, No. 3, 1914.]

THE EXCRETION OF NITROGEN IN FEVER.*

BY N. C. SHARPE, M.B., AND K. M. B. SIMON, M.B.

(From the Department of Pathological Chemistry of the University of Toronto, Toronto.)

That there is no qualitative difference between the metabolism of fever and normal metabolism, and no ground for believing that proteins are broken down in infective fevers by the action of toxins upon the protoplasm of the cell, are the conclusions to which Krehl and Grafe (1) have come as the result of work recently done in the laboratories of the Heidelberg clinic.

The evidence adduced by Grafe (2) upon the meaning of the increased nitrogen excretion in fever is partly derived from measurements of the respiratory exchange in fever patients on a fluid diet yielding about 1,200 calories and containing about 5 to 6 gm. of nitrogen, and calculation, from the nitrogen output, of the ratio of the energy derived from protein oxidation to the total amount of energy liberated. This ratio he finds to be similar to that observed in starvation. Partly too (3), from the fact that, if a sufficiently liberal estimate of the energy needs of such patients be made, about 50 calories per kilo, and this amount be supplied in the food, mostly in the form of carbohydrate, nitrogenous equilibrium may be maintained in fever, in most cases when less than 1.4 gm. of protein per kilo of body-weight are given. This amounts, he argues, to proof that the loss of nitrogen often observed in fever is due to the increased evolution of energy in fever and the diminished intake of food. He claims support for this view also from the results obtained by Shaffer and Coleman (4), who prevented loss of nitrogen in typhoid fever by administering very large quantities of carbohydrate. More recently Pfannmüller (5) has succeeded in reducing the output of nitrogen in fever as much as 40 per cent. by adding to an otherwise constant diet 500 gm. of sugar, whereas in patients on this diet whose temperature was normal the addition of this amount of sugar reduced the nitrogen output only about 10 per cent., showing that the largest part of the loss of nitrogen in fever is due to the deficiency of carbohydrate.

It seems clearly established at any rate from all these results that when a liberal supply of carbohydrate can be given it may reduce the destruction of tissue protein in fever. But this result does not necessarily imply that the destruction of protein that would otherwise take place is due merely to a deficiency of calories in the food;

---

* Received for publication, June 19, 1914.

the presence of large quantities of carbohydrate may affect the course of chemical processes in the body in other ways than merely by furnishing material that is easily oxidized. It is not altogether in harmony with what we know that it should be necessary to supply 50 calories per kilo to a patient in bed even if he is in fever. There is a fair consensus of evidence that the respiratory exchange is increased in fever often 20 or possibly 30 per cent., seldom more than that, and also that 30 calories per kilo is a sufficient supply for a patient lying in bed; if 30 per cent. be added to meet the calls due to the fever, 40 calories per kilo should theoretically suffice in most cases of fever. In Shaffer and Coleman's experiments, it was necessary so to drench the patients with carbohydrate that the calories supplied amounted to 80 or even 100 per kilo.

In some ways the most valuable study of metabolism in fever that has yet been carried out is that of Likhatcheff and Avroroff (6). A woman suffering from malaria was examined in the calorimeter on days on which attacks occurred, and also when her temperature remained normal, but conditions were otherwise similar. With a normal temperature her heat production was 32 calories per kilo per day. On a day when her temperature rose about 3° C. to 39.3° heat production rose to a little more than 35 calories per kilo.

It is difficult to see why, in order to prevent so small an increased evolution of energy as this resulting in the destruction of proteins in the body, it may be necessary to supply energy at a rate two or even three times as great as this, most of it in the form of carbohydrate, if the protein is attacked merely because there is not carbohydrate available.[1] The experiments of Kocher (7) recently reported showed that a diet of 70 calories per kilo containing only 2 gm. of nitrogen, which in patients whose temperature was normal gave an output of nitrogen amounting only to 3 gm., resulted in the loss of much larger amounts of nitrogen, up to more than 20 gm., in the case of patients in fever.

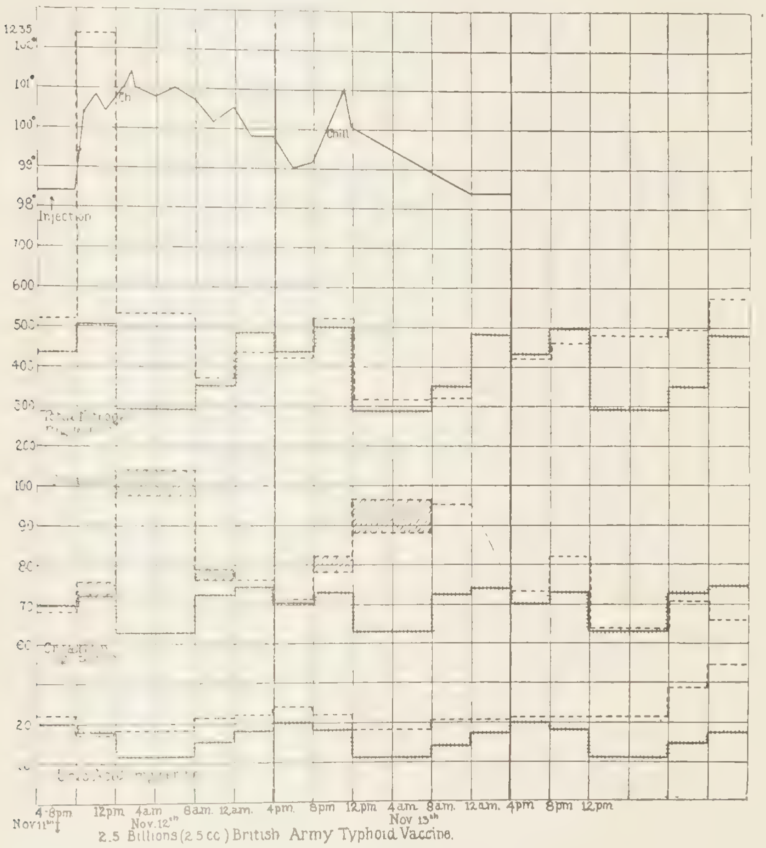
---

[1] It is true that this may not be a fair way of looking at the case in point. The energy output for the day on which the fever occurred amounted, when reckoned for the twenty-four hours, to only 10 per cent. more than on a normal day. But a valuable feature of the experiment is that the effect is followed not merely by the coarse method of twenty-four-hour periods, as is the case with most work on fever. A fine adjustment to two-hour periods was made use of, and it becomes apparent that between the hours of 3 and 6 A. M. the rate of heat production rose to 110 calories per hour when at these hours with a normal temperature it would have been only about 60 calories per hour. Such a sudden short burst of racing heat production might possibly be supposed to overstrain the immediately available supply of carbohydrate, and temporarily necessitate recourse to protein material; only it must be remembered that still greater departures from the usual rate of heat production, in muscular exertions, can be borne, as is well known, without any such result.

The principal difficulty in the study of metabolism in fever consists in the establishment of proper controls with which to compare the abnormal change. Controls obtained from the same individual during convalescence are not always necessarily satisfactory, and they are generally the best that can be obtained. The ideal condition would be to have the subject under examination for some days before the onset of the fever and to be able to keep the diet constant all the time. In the case of malaria this might be possible, but hardly in that of any other disease. But something may be done with the febrile reaction induced by the injection of toxins. Two experiments of this kind done on the same subject with typhoid vaccine were reported by Leathes in 1907 (8), and we have done two more on two other subjects with typhoid vaccine and four on four different subjects with tuberculin following essentially the method employed by him; we have also examined a patient suffering from malaria.

The procedure adopted consists in establishing the normal rates at which total nitrogen, creatinin, and uric acid are excreted by the subject at different periods of the day, by collecting the urine at fixed hours several times daily for several days on a constant diet. After a week the average output for each period of the day is calculated and plotted on squared paper representing the normal rate of excretion for that individual on that diet. The injection is timed so as to bring on the reaction in the evening and the maximum temperature in the night so that the intake of food is not interfered with at all and the disturbance of nutrition that it is otherwise so hard to allow for is entirely eliminated. On the next day the temperature has generally fallen; no meals are missed and the diet remains the same. The rates at which total nitrogen, creatinin, and uric acid are excreted during each period following the injection are then plotted on the same paper with the normal average rates previously obtained, and any departure from the normal becomes at once apparent. In this way the changes effected can be followed more minutely, and pronounced departures from the normal course can be detected, which being of short duration might otherwise be lost sight of in averaging up the whole output for twenty-four hours.

The results obtained are presented in the form of curves in text-figures 1 to 8. Text-figure 3 gives for comparison the results of one of the experiments referred to above and previously reported by Leathes. The abscissæ represent time, divided into periods corresponding to the times at which the samples of urine for analysis were collected, generally every four hours. The ordinates give the hourly rate of excretion of either total nitrogen, creatinin, or uric acid, as indicated on the scale at the left hand side. In each case the dotted interrupted line shows the hourly rates for each period



TEXT-FIG. I.[2] Case I. Typhoid vaccine.

---

[2] In the text-figures, the black lines represent the normal rate of excretion in mg. per hour of uric acid, creatinin, and total nitrogen, being the average of several days under fixed conditions, divided in four-hour periods. The dotted lines represent the rate in mg. per hour following the injection.

following the injection; the average normal hourly rates calculated from six to eight days on the same diet preceding the injection are shown as continuous lines (in text-figure 1 these lines have cross markings), and being normal averages repeat themselves every twenty-four hours. The charts for convenience have black vertical lines running through them dividing them into periods of twenty-four hours. The temperature curve is given so as to show the relation of the changes in the rates of excretion to the changes in body temperature.

The diet was in most cases milk and bread and butter; in some cases it included eggs and cheese or fruit. No meat or meat broths were included in any case. In all cases as far as possible the composition and amount of food and liquid taken were the same on each day of any given experiment and they were taken at the same hours throughout that experiment.

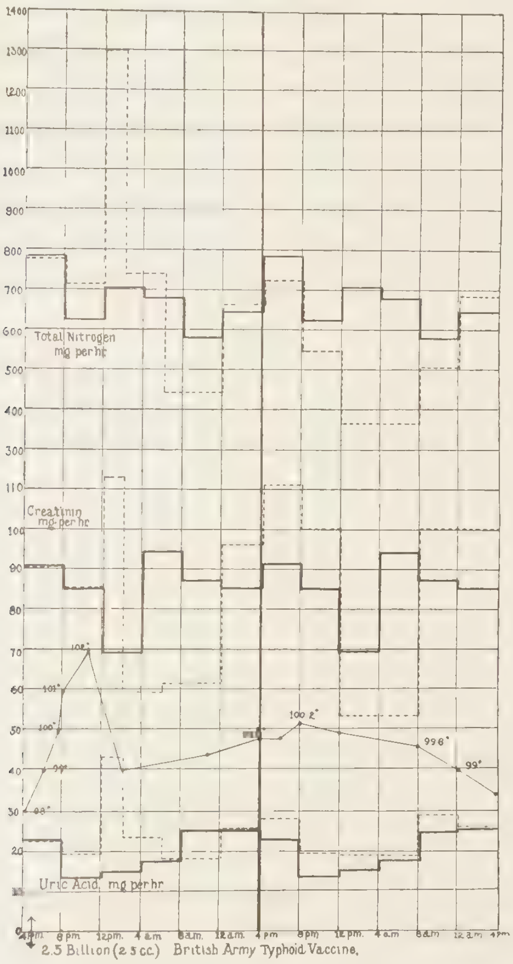
Creatinin was estimated by Folin's colorimetric method with bichromate solution for standard; uric acid by his modification of Hopkins' method; total nitrogen by Kjeldahl's method. Creatin was looked for in all cases except the experiment charted in text-figure 3; when found the amount is indicated by a shaded area in the creatinin curve.

The time at which the injection was given is marked by an arrow on the chart.

If now the first three curves which give the results of experiments with typhoid vaccine are consulted, it will be observed that they agree in showing a sharp rise in the excretion rates synchronizing closely with the rise of temperature, and that they agree also in showing a greater change in the excretion of uric acid than of either creatinin or total nitrogen. When the reaction occurs the excretion of uric acid in case 1 is increased more than 60 per cent. for eight hours, and for more than twenty-four hours after that remains uniformly increased. In case 2 it is nearly trebled for two hours, and in case 3 it is more than trebled for eight hours and remains above the normal for several hours after that.

In table I the quantities excreted in twenty-four-hour periods after the injection, as compared with the normal twenty-four-hour average, are given. It illustrates how a marked reaction may be missed if the whole of the urine for twenty-four hours is analyzed together, most notably in case 2.

In case 1 the uric acid in the blood was estimated on three days at the same hour by Folin's colorimetric method. On two days before the injection the amount found in double estimations was 0.82 and 0.81 of a milligram per 100 cubic centimeters on the first, and 0.80 and 0.77 on the second. On the day when the injection was given,



TEXT-FIG. 2. Case 2. Typhoid vaccine.

TABLE I.

Typhoid Vaccine.

Total Excretion in Periods of Twenty-Four Hours.

	Total nitrogen.		Uric acid.		Creatinin.	
	Gm.	± per cent.	Gm.	± per cent.	Gm.	± per cent.
Case 1. N. C. S.						
Normal average	8.9		0.36		1.64[3]	
1st 24 hrs. after injection	14.4	+61	0.48	+33	2.02[4]	+23
2d 24 hrs. after injection	9.2	+3	0.48	+33	2.05[4]	+25
3d 24 hrs. after injection	11.8	+33	0.60	+67	1.68[3]	+2
Case 2. K. M. B. S.						
Normal average	16.1		0.46		2.10[3]	
1st 24 hrs. after injection	16.8	+4	0.55	+20	1.90[3]	−10
2d 24 hrs. after injection	13.0	−20	0.56	+21	2.10[3]	
Case 3. J. L.						
Normal average	7.6		0.44		2.07[5]	
1st 24 hrs. after injection	9.8	+28	0.71	+61	2.24[5]	+8

and seven and a half hours after it, the amount found was 1.75 milligrams per 100 cubic centimeters. In the urine that had been excreted during those hours the uric acid corresponded, as may be seen in text-figure 1, to the amount excreted at that time on normal days; but immediately after that the rate of excretion was increased and remained so for more than forty-eight hours. This result shows that the increased excretion cannot be accounted for merely by increased renal activity, but corresponds to a really increased production.

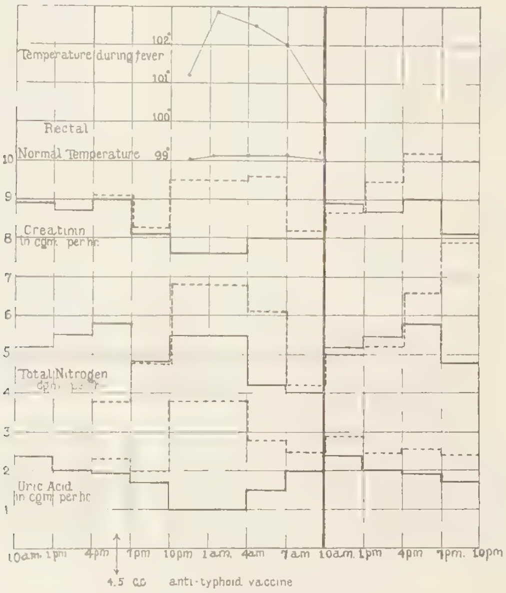
We find it difficult to reconcile entirely such results as these with the conclusions to which Krehl and Grafe have come with regard to the interpretation of the increased nitrogen excretion in fever. It is difficult to see how these marked reactions can be put down to a sudden deficiency of carbohydrate; the subjects were in good condition, well nourished, strong, healthy, and capable at any moment like other healthy subjects of doubling or trebling their ordinary rate of discharge of energy by muscular exertions without showing any such change in the nitrogen excretion. Moreover, it appears to us that there is evidence of a qualitative change in metabolism during

[3] No creatin found.

[4] Including creatin.

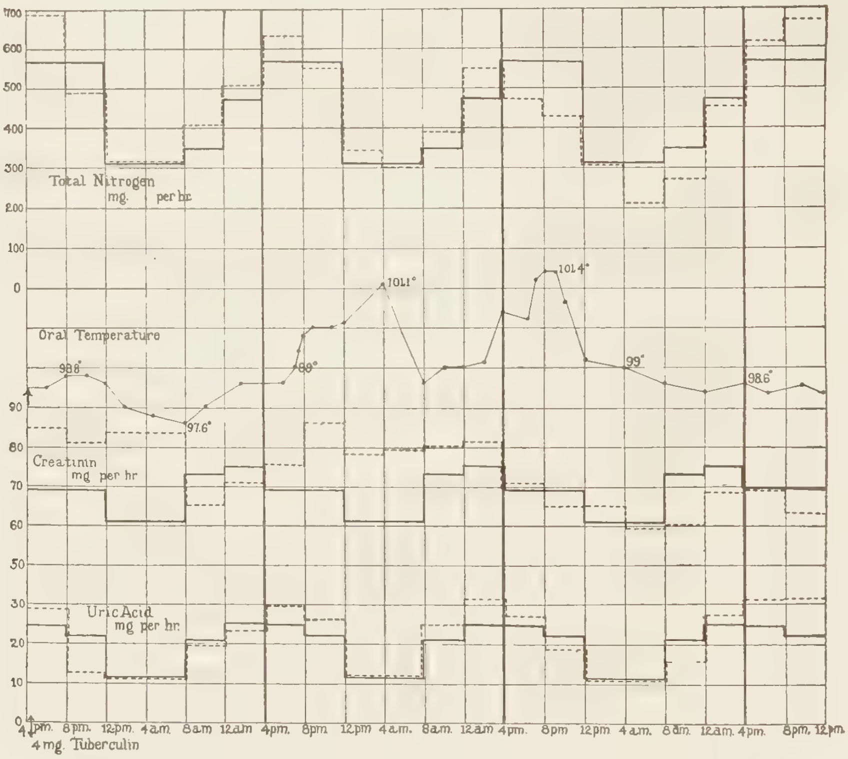
[5] Creatin not estimated.

a febrile reaction to be detected also in the way in which the uric acid is more affected than other forms of nitrogenous excreta. The reaction of the body to the typhoid toxin seems to consist not in a rise of temperature due, so far as it is due to increased heat production, to a general acceleration of metabolism qualitatively the same as that which was proceeding before the reaction was excited, but to chemical processes of a special kind. Whether these processes are merely certain special factors in normal metabolism that are necessarily exaggerated in the heightened activities, nervous or muscular or of whatever nature they may be, that are involved in the reaction, or whether they are toxogenic and due to the damage done to cell protoplasm by the toxin, it is not possible from these data alone to decide. Possibly a comparison of the results of the injection of typhoid vaccine with the changes brought about by tuberculin, to which we now turn, may help to decide this question.



TEXT-FIG. 3. Case 3. British Army typhoid vaccine.

The results of tuberculin injections when studied in this way in four subjects, two of them normal, one slightly, the fourth severely infected, are given in text-figures 4 to 7 and table II. It is clear that the reaction to this toxin is slower than the one already dealt with. Except in the case of the subject who was severely infected (case 7), about twenty-four hours elapse before the temperature rises, and the rise of temperature when it occurs tends to be less. The abrupt change in the rates at which nitrogenous excreta are turned out, that accompanies the prompt rise of temperature after typhoid vaccination, is missed in cases 4, 5, and 6, which reacted slowly, and is noticeable in case 7, which reacted more rapidly to tuberculin, only after the first injection. But in that instance the rise of temperature was little more than 1° F., and similar temperature reactions to a second and third dose, though these doses were larger, were accompanied by less definite changes in the excretion.



TEXT-FIG. 4. Case 4. Tuberculin. Normal subject.

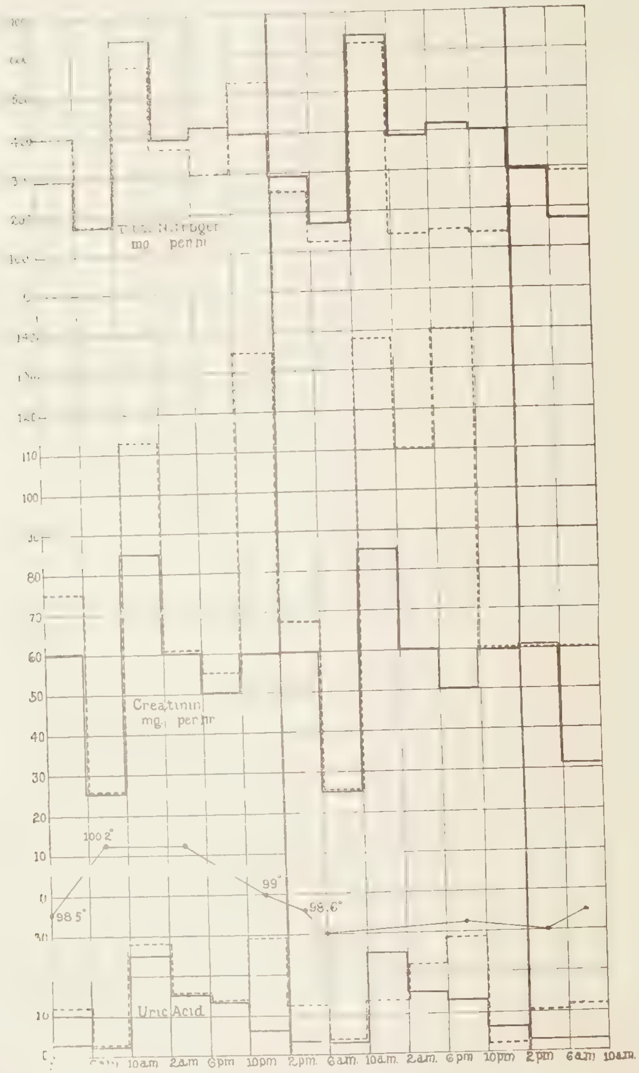
TABLE II.

Tuberculin.

Total Excretion in Periods of Twenty-Four Hours.

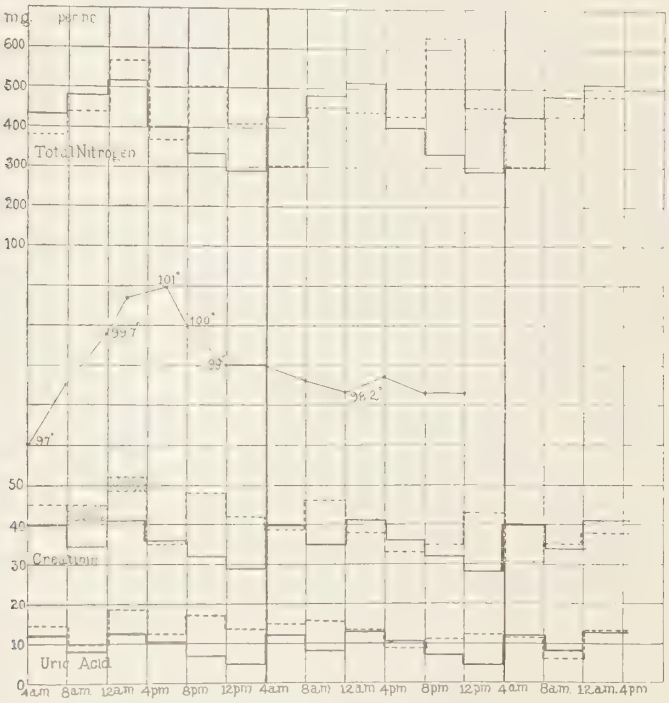
	Total nitrogen.		Uric acid.		Creatinin.	
	Gm.	± per cent.	Gm.	± per cent.	Gm.	± per cent.
Case 4. N. C. S. (No tuberculosis)						
Normal average	10.2		0.45		1.6	
1st 24 hrs. after injection	10.4	+ 2	0.41		1.8	+13
2d 24 hrs. after injection	10.9	+ 7	0.52	+15	1.9	+19
3d 24 hrs. after injection	8.3	19	0.41	− 9	1.4	−13
Case 5. C. L. (No tuberculosis)						
Normal average	8.9		0.26		1.3	
1st 24 hrs. after injection	12.6	+42	0.46	+77	1.9	+46
2d 24 hrs. after injection	8.8		0.26		1.8	+40
Case 6. W. P. (Slight tuberculosis)						
Normal average	9.8		0.22		0.84	
1st 24 hrs. after injection	10.5	+ 7	0.30	+36	1.10	+31
2d 24 hrs. after injection	9.9		0.34	+55	0.94	+12
3d 24 hrs. after injection	10.7	+ 9	0.24	+ 9	0.85	
Case 7. R. K. (Marked tuberculosis)						
Normal average	12.7		0.46		1.22	
1st 24 hrs. after injection	13.4	+ 5	0.50	+ 9	1.24	
2d 24 hrs. after injection	12.6		0.46		12 5	

The change in the output for twenty-four-hour periods shown in table II is considerable in cases 4, 5, and 6 in regard to creatinin, in cases 5 and 6, but less so in case 4 in regard to uric acid, in case 5 and less so in case 6, in regard to total nitrogen. Case 4 showing the reaction to tuberculin in the same subject as case 1 to typhoid vaccine is particularly instructive, especially as the temperature rose to the same level in both experiments, and the marked effect on the output of uric acid in the reaction of this subject to typhoid vaccine is missed in the reaction to tuberculin. The reaction to typhoid was accompanied by definite rigors, that to tuberculin was not. There does not therefore appear to be the same uniformity in the reaction of different subjects to tuberculin as in the reaction to typhoid vaccine. And the most striking feature in the reaction to typhoid vaccine, the sudden change in output of all the forms of nitrogenous excreta investigated, accompanying the rapid rise of temperature, is not to be observed with the slower reaction to tuberculin. But in the reaction to tuberculin as in that to typhoid vaccine the changes that occur in the output of nitrogenous substances are such as to make it diffi-



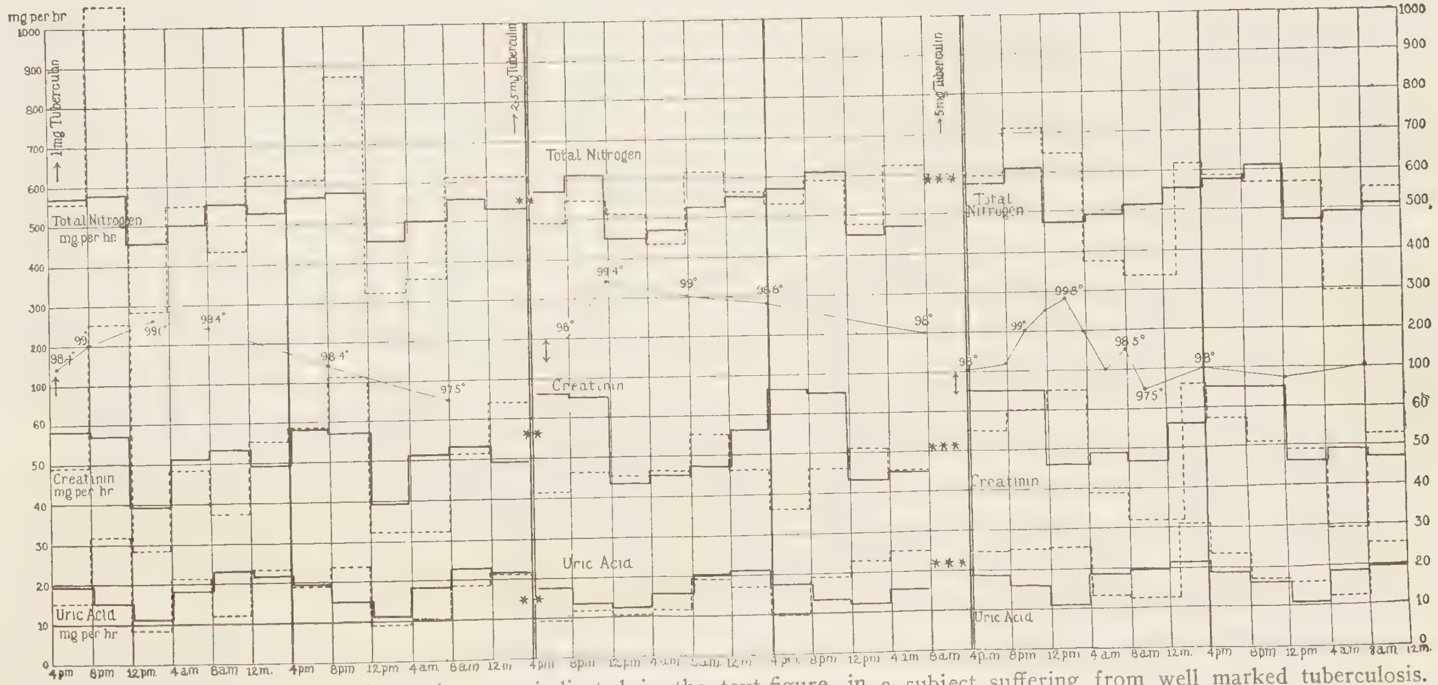
TEXT-FIG. 5. Case 5. Tuberculin, 1 mg. eighteen hours before. Normal subject.

cult to concur in the conclusion that there is no qualitative difference between the metabolism in fever and normal metabolism. Whether the other fact which these experiments bring out, namely that the effects of the two toxins are different from each other in certain respects, should be interpreted as showing that the increased output is not due to increased activities involved in the process of raising the body temperature, and involved therefore alike whichever toxin was used, but is due rather to the two poisons attacking the cell protoplasm in ways that are different, though both of them cause a toxogenic nitrogen excretion, this is a question which the data are not yet sufficient to determine.



Text-Fig. 6. Case 6. Tuberculin, 2 mg. sixteen hours before. Subject suffering from slight tuberculosis.

Finally there remains the case of malaria. In this instance, however, it was impossible to get normal control figures in the way



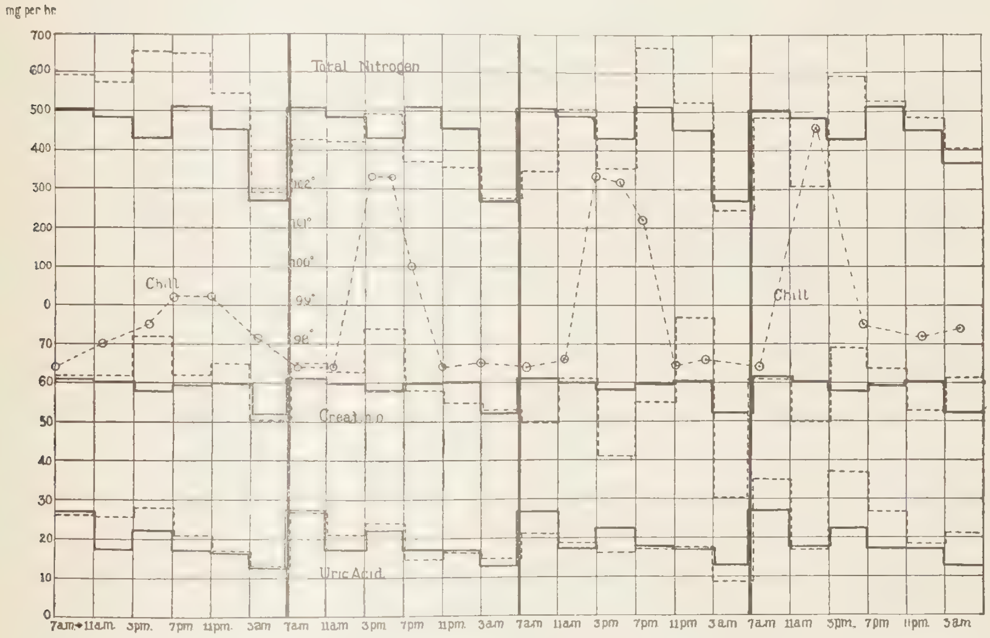
Text-Fig. 7. Case 7. Tuberculin, 3 doses as indicated in the text-figure, in a subject suffering from well marked tuberculosis.

TABLE III.

Malaria (Double Tertian).

Case 8. V. K.	Total nitrogen.		Uric acid.		Creatinin.		Maximum temperature F.	Remarks.
	Gm.	± per cent.	Gm.	± per cent.	Gm.	± per cent.		
1st dy......	13.2	+26	0.52	+16	1.49	+ 6	99.2°	Rigor.
2d dy......	9.4	−10	0.48	+ 7	1.47	+ 5	102.3°	
3d dy......	10.5	± 0	0.39	−13	1.26	− 10	102.3°	
4th dy.....	11.2	+ 7	0.62	+38	1.43	+ 2	103.6°	Rigor.
5th dy.....	10.5		0.45		1.40		98.2°	Quinin.

The percentage variations are calculated with reference to the 5th day as standard, when quinin was given and there was no rise of temperature.



TEXT-FIG. 8. Case 8. Malaria.

in which this was done with the other cases. The patient had had several days of fever before he came under observation. Analyses were carried out on four days on which there was fever, on two of them preceded by rigors, and on a fifth day when administration of quinin prevented both rigor and fever. The figures obtained on the fifth day are charted as the continuous line and are given for comparison with those for each twenty-four-hour period on which fever occurred. As far as the available data go they show higher figures for uric acid and total nitrogen on the days on which there was rigor than on any of the others, and the fact that the effect of the reaction on the excretion of nitrogenous substances is different from that observed with the typhoid vaccine and also from that after tuberculin is perhaps in favor of the view that different pyrogenic toxins affect the excretion of nitrogen in different ways because the damage done to the cell protoplasm is different. But the lack of satisfactory controls makes the figures obtained from this case less significant (table III).

CONCLUSIONS.

The rise of temperature following typhoid vaccination is accompanied by a marked increase in the output of total nitrogen, creatinin, and especially of uric acid.

The reaction to tuberculin is slower, but is also accompanied by an increased output of nitrogenous substances, that is, however, less uniform and generally less pronounced.

In a case of malaria there was a tendency for the rise of temperature to be accompanied by an increased output of total nitrogen and less uniformly of creatinin; on two days on which the fever was preceded by rigor the output of uric acid was increased.

BIBLIOGRAPHY.

1. Krehl, L., *Verhandl. d. deutsch. Cong. f. inn. Med.*, 1913, xxx, 26. Grafe, A., *idem*, p. 57.
2. Grafe, E., *Deutsch. Arch. f. klin. Med.*, 1910, ci, 209.
3. Rolland, A., *Deutsch. Arch. f. klin. Med.*, 1912, cvii, 440.
4. Shaffer, P. A., and Coleman, W., *Arch. Int. Med.*, 1909, iv, 538.
5. Pfannmüller, *Deutsch. Arch. f. klin. Med.*, 1913, cxiii, 100.
6. Likhatcheff, A. A., and Avroroff, P. P., *Reports of the Imperial Military Medical Academy, St. Petersburg*, 1902, v, 1.
7. Kocher, R. A., *Deutsch. Arch. f. klin. Med.*, 1914, cxv, 82.
8. Leathes, J. B., *Jour. Physiol.*, 1907, xxxv, 205.

Journal of Pathology and Bacteriology, Vol. XIX.

ON FATTY CHANGES IN THE LIVER, HEART, AND KIDNEY.[1]

By C. G. Imrie, M.B., *Medical Research Fellow, University of Toronto.*

From the Department of Pathological Chemistry, University of Toronto.

In a paper by Hartley and Mavrogordato,[2] attention was first called to the fact that when the amount of fat in the liver is increased the amount of unsaturated acids in that fat as determined by their iodine value is diminished.

This fact has been repeatedly confirmed and holds for other species besides man; when the liver contains what may be regarded as a normal amount of fat, yielding fatty acids after saponification amounting to less or little more than 3 per cent. of the weight of the fresh tissue, then the iodine value of these acids will be about 135; whereas when more is present this value is lower, till in the highest degrees of fatty change, when the fatty acids obtained may amount to 20 per cent. of the fresh weight, the iodine value is almost as low as that of the acids obtained on saponifying adipose tissue. This, in conjunction with certain other facts, gave rise to the following comment:—"It is difficult to see how this is to be explained except by supposing that when there is an excessive amount of fat in the liver it is because an excessive amount of the stored fat of the body has been brought to the liver. And the inference comes ready to hand that a normal liver too has fat brought to it composed largely of saturated acids, but being normal it is able to deal with this fat, converting the saturated acids into less saturated ones."[3]

In the same place reference is made to the case of the heart and kidney, which are also liable to fatty changes, but in which sufficient determinations of this nature had not been made, in order to say whether in them too a similar relationship holds between the amount of fat and of unsaturated acid, though it was already clear that variations on the scale upon which they are found in the liver did not occur in them.

---

[1] Received May 8, 1914.

[2] *Journ. Path. and Bacteriol.*, Cambridge, 1908, vol. xii. p. 371.

[3] Harvey Lecture on the "Functions of the Liver in relation to the Metabolism of Fat," by J. B. Leathes, *Lancet*, London, February 27, 1909.

The fatty acids obtained from the normal heart and kidney, like those obtained from the normal liver, differ from those in the fat of connective tissue in that they consist largely of acids more unsaturated than oleic acid; their iodine value is 135 or more, while that of the acids of adipose tissue is in man about 65, and that of oleic acid is 90. If an organ, therefore, becomes fatty because of an access of connective-tissue fat, the iodine value may be expected to fall in proportion to the amount of connective-tissue fat imported.

In a considerable number of cases, mostly from the Toronto General Hospital, the liver, heart, and kidneys were examined in the same way. Weighed portions freed from connective tissue were heated with potash till liquefied, alcohol added, and the heating continued for half an hour; the fatty acids were precipitated from the soap solution by sulphuric acid, and taken up in a measured quantity of petroleum ether of which a fraction was evaporated, and the residue weighed and taken for the determination of its iodine value by the method of Wijs.[1]

The results have been grouped in four tables. In the first two the cases are arranged in order of the amount of fat found in the liver; in the others, in order of the amount found in the heart and kidney respectively. In each table the group which contained the smallest amount of fat was taken as a standard, together with the average iodine value for this group. And using this standard a curve was constructed for each organ with percentage of fat represented in the abscissæ, and iodine values in the ordinates, so as to show the iodine value that would correspond to any percentage of fat on the supposition that any additional amount of fat found in such an organ was imported connective-tissue fat. In the cases of the liver, for instance, there were eleven cases averaging 2·8 per cent., with the iodine value 136. Taking these values as the standard for this organ, then if an equal quantity of connective-tissue fat with the iodine value 65 were imported, we should have 5·6 per cent., with the iodine value 100·5; if double the amount, then 8·4 per cent., with the iodine value 89, and so on. The curve so obtained is drawn in a continuous line in Fig. 1. It therefore represents the conditions that would obtain if importation of connective-tissue fat were the only factor in the fatty change.

The average observed values for the different groups in Table I., with their average iodine values, are shown in the diagram as points surrounded by circles. It is clear that the correspondence between the observed values and the curve is so close that the importation of connective-tissue fat or, what amounts to the same on ordinary diets, of fat absorbed from the intestine, seems to be the main factor determining fatty change in the liver. But it is to be noticed that as the percentage increases up to 6 per cent., or a little more than double the standard amount of fat, the observed values tend to be above the curve, but with three times the standard amount or more than that they tend to be below the curve. If the function of the liver is, as the articles referred to above contend, to desaturate fatty acids, then it would appear that this function is stimulated by amounts of fat up to about double the standard amount, increasingly embarrassed by amounts larger than that.

---

[1] The details of the method and precautions taken were those prescribed in Longmans' biochemical monograph on The Fats, by J. B. Leathes, 1910. Cholesterol, when it was estimated, was estimated in a separate portion; all the figures used in this paper refer to the extract composed of fatty acids mixed with from 10 to 15 per cent. of cholesterol.

In Table II. the results of the examination of the organs of a few infants and fœtuses are given separately; the figures for the amount of fat in the liver appear to be lower than in adults, and accordingly, though the data are obviously too few in number, they have been treated separately and referred to the dotted curve in Fig. 1, the standard for which is the mean of the two cases where the iodine value was about the same as the standard for adults, and which also represents the conditions that would be required on the supposition that any access of fat above the standard amount would be connective-tissue fat with the same iodine value as in adults. The observed values follow the course of the theoretical curve sufficiently closely to justify the expectation that with a larger number of data the correspondence with that curve would be as close as in the case of the adult livers.

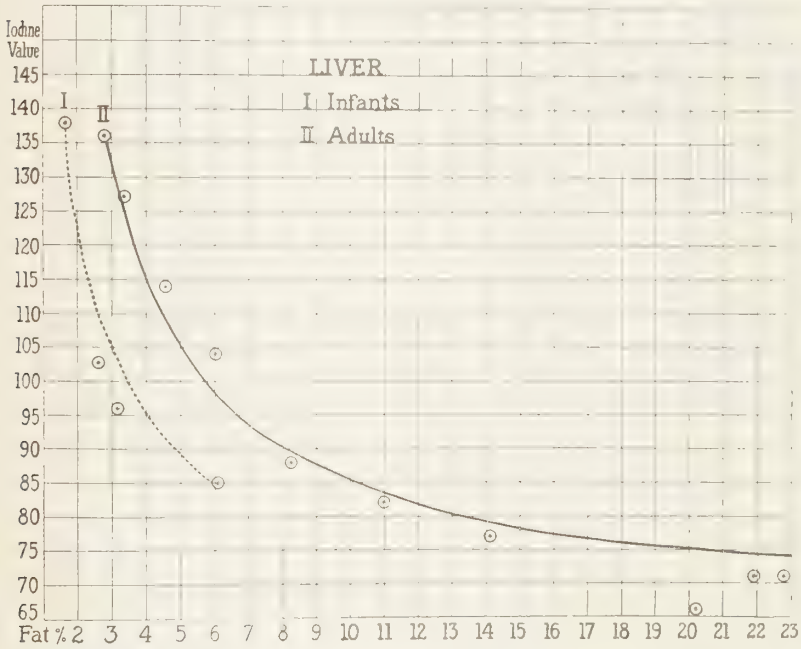


Fig. 1.

In the case of the heart and kidney the first point to be noted appears in Table I. The cases, grouped there in order according to the amount of fat in the liver, and exhibiting a gradual increase in iodine value from group to group as the amount of fat in the liver approaches the standard value, show no such orderly sequence in the other organs in these respects. The largest average amount of fat in the heart, for instance, occurs in the fifth group, and the next largest in the fourth and seventh, and the average iodine value of the fatty acids from the heart in the third group of cases is practically the same as in the last group; and so too with the kidney, so that it is clear that the condition of the fat in the liver is not what determines the condition of fat in these organs. If they normally take up fat that has been desaturated in the liver, when fat is accumulating in the liver, they do not appear to take up, because that is so, the unaltered fat from the blood; at least such fat is not necessarily found in them in increased amounts. A liver in which the unaltered fat is accumulating may nevertheless be forwarding a normal amount of normally desaturated fat. The accumulation in the liver is not necessarily caused only by failure of the liver to do its work; it may be due to excessive activity in the mobilisation of fat from the storage depots, and to the fact that the liver does not send out the fat it has received till it has been desaturated. But when the figures obtained for the heart and kidney are arranged, as in Tables III. and IV., in groups in order of the amount of fat contained in these organs severally, it is observed that here too the average iodine value for the groups increases as the amount of fat approaches that found in the group containing least fat. So that there is a tendency for the fat in these organs also, when they contain more than usual, to approach in some degree to the type of connective-tissue fat.

When, however, curves are constructed, as has been done in Fig. 2, similar to the curve for the liver in Fig. 1, taking as standard value the average percentage and iodine values observed in the group with the smallest amount of fat, and representing the conditions that would obtain if all fat above that amount were imported connective-tissue fat with the iodine value 65, the average observed values are all found to lie above these curves; in other words, the iodine value does not sink, as it does in the liver, as much as it would if all the additional fat were imported direct from the connective tissues. The discrepancy between the observed values and the values calculated on that basis increases as the amount of fat increases. The fatty changes in these organs therefore do not admit of the simple explanation that is so clearly indicated in the case of the liver, that all additional fat is imported from the connective tissues. Some of it may be, but the more fat the organ contains the smaller is the proportion of the additional fat that is of that nature.

Two ideas with regard to the fatty changes in heart and kidney are compatible with what is known: one is that an organ such as the heart, and probably the kidney, in which fat is oxidised to supply energy, may when diseased lose this power, and in that case become encumbered with a supply of the material that it has lost the power of burning up. If there was a tendency for such fat lying idle to revert to the saturated connective-tissue type, then a condition would result similar to that which the figures reveal.

The other is that the complex fatty substances containing phosphorus degenerate in a diseased organ as they do in a divided nerve. If the importation of desaturated fat from the liver to replace the degenerated fat went on as usual, the amount of fat would increase in the organ, and with a certain amount of reversion of the degeneration products to fat of the saturated type, then, too, a condition would be established similar to that observed.

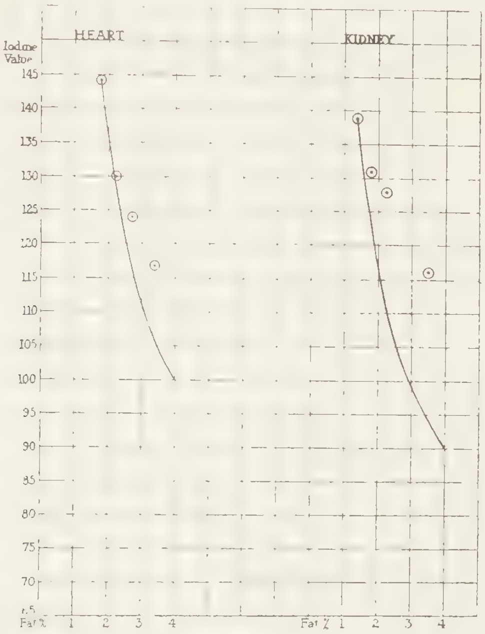


FIG. 2.

The two points that the figures obtained seem to make certain are (1) that the accumulation of fat in the liver does not determine the condition of the heart or kidney; (2) that while in the liver fatty change is certainly an accumulation of connective-tissue fat, in the heart and kidney some other factor determines, or at least co-operates in determining, the change.

The histological protocols were all checked by Professor J. J. Mackenzie, to whom I am much indebted for this assistance. The rest of the work was carried out under the direction of Professor J. B. Leathes.

[TABLES

TABLE I.—*Cases (Adult) grouped according to Amount of Fat in Liver.*

	Case No.	Diagnosis.	Liver.		Heart.		Kidney.	
			Per cent.	Iodine Value.	Per cent.	Iodine Value.	Per cent.	Iodine Value.
(A) More than 20 per cent	40	Alcoholism	21·90	71	2·97	128	2·54	126
	(A1)	Tuberculosis	22·80	71	...	...	...	...
	(A2)	HCl poisoning	20·20	66	...	...	...	...
(B) More than 12 per cent., less than 16 per cent.	35	Pneumonia	15·41	76	1·60	133	1·92	137
	23	Tuberculosis	12·81	78	2·80	118	2·41	119
		Average .	**14·11**	**77**	**2·20**	**126**	**2·17**	**128**
(C) More than 10 per cent., less than 12 per cent.	30	Addison	11·75	83	2·04	121	3·25	126
	7	Coroner's Case	11·19	84	1·89	141	1·70	137
	24	Chronic pancreatitis	10·64	79	2·09	144	2·27	129
	29	Puerperal sepsis	10·31	81	1·94	147	1·84	137
		Average .	**10·97**	**82**	**1·99**	**138**	**2·27**	**132**
(D) More than 7·5 per cent.; less than 10 per cent.	31	Pneumonia	8·31	94	1·86	152	1·92	123
	8	,,	8·17	87	1·99	131	1·79	130
	41	Cholecystitis	7·83	85	3·35	119	...	...
	(A3)	Acute pancreatitis	8·80	86	...	...	...	...
	(A4)	Hæm. pancreatitis	8·54	86	...	...	...	...
	(A5)	Acute yellow atrophy	8·10	88	...	...	...	...
		Average . .	**8·29**	**88**	**2·40**	**134**	**1·86**	**127**
(E) More than 5 per cent.; less than 7·5 per cent.	39	Malign. endocarditis	6·66	100	2·59	133	2·40	117
	25	Typhoid	6·37	105	1·77	155	1·68	141
	36	Diabetes; lipæmia	5·93	104	2·36	132	3·72	105
	3	Pernicious anæmia	5·69	107	3·77	109	1·55	132
	(A6)	Tuberculous meningitis	5·67	103	...	...	...	...
		Average . .	**6·06**	**104**	**2·62**	**132**	**2·34**	**124**

Table I.—continued

	Case No.	Diagnosis.	Liver.		Heart.		Kidney.	
			Per cent.	Iodine Value.	Per cent.	Iodine Value.	Per cent.	Iodine Value.
(F) More than 4 per cent.; less than 5 per cent.	38	Pernicious anæmia	4·75	109	2·01	135	1·90	131
	18	Typhoid	4·65	116	1·92	142	1·80	124
	12	Fractured skull	4·49	...	1·66	144	1·85	135
	2	Pernicious anæmia	4·14	117	2·62	112	1·84	111
		Average . .	**4·51**	**114**	**2·05**	**133**	**1·85**	**125**
(G) More than 3 and less than 4 per cent.	42	Acute peritonitis	3·80	118	2·20	138	2·03	146
	17	Hydroquinone poisoning	3·45	126	3·33	120	1·76	127
	21	Toxæmia of pregnancy	3·27	115	1·80	143	1·48	...
	22	Carcinoma of pancreas	3·21	131	3·11	122	1·38	125
	33	Miliary tubercle	3·14	140	1·61	159	1·60	143
	32	Pneumonia	3·02	131	2·18	118	...	...
		Average .	**3·32**	**127**	**2·37**	**133**	**1·65**	**135**
(H) Less than 3 per cent.	11	Typhoid	2·99	120	...	...	1·47	133
	10	Pneumonia	2·98	137	1·88	142	1·84	125
	27	Gastro-enterostomy	2·95	139	1·92	151	1·43	157
	26	Puerperal fever	2·90	133	1·40	141	1·31	137
	1	...	2·89	138	2·19	127	1·44	139
	6	Pneumonia	2·86	159	2·43	136	2·01	129
	16	,,	2·75	139	2·52	131	1·32	138
	13	Endocarditis	2·74	127	1·82	124	1·67	130
	37	Septicæmia	2·66	134	1·83	156	1·22	142
	19	Tuberculosis	2·54	134	1·57	140	1·84	123
	9	Typhoid	2·46	131	1·65	141	1·52	134
		Average .	**2·80**	**136**	**1·92**	**139**	**1·55**	**137**

TABLE II.—*Organs obtained from Infants and the Fœtus.*

Case No.	Age.	Liver.		Heart.		Kidney.	
		Per cent.	Iodine Value.	Per cent.	Iodine Value.	Per cent.	Iodine Value.
4	6 months	6·09	85	2·87	97	1·73	128
5	2 days	3·18	96	2·19	108	2·41	108
20	7th month fœtus	2·58	103	1·26	132	1·71	138
15	2 days	1·90	143	1·21	162	1·31	142
14	6th month fœtus	1·38	132	1·32	148	1·36	125

TABLE III.—*Cases grouped according to Amount of Fat in the Heart (Adults).*

	Case No.	Per cent. of Fat in Heart.	Iodine Value.	Histological Notes.
A . .	3	3·77	109	+ + +
	41	3·35	119	+ + + not fatty to N.E.
	17	3·33	120	+ + +
	22	3·11	122	+ + +
Average of 4 cases with more than 3 per cent. in heart.		**3·39**	**118**	
B . .	40	2·97	128	+ + +
	23	2·80	118	+ + +
	2	2·62	112	+ + +
	39	2·59	133	+ + +
	16	2·52	131	+ +
Average of 5 cases with more than 2·5 per cent. in heart.		**2·70**	**124**	
C . .	6	2·43	136	+ +
	36	2·36	132	+
	42	2·20	138	+ +
	28	2·20	127	+
	1	2·19	127	No sections.
	32	2·18	118	+ + +
	24	2·09	144	+
	30	2·04	121	+
	38	2·01	135	No sections.
Average of 9 cases with more than 2 per cent. in heart.		**2·19**	**131**	
D . .	8	1·99	131	Brown atrophy.
	29	1·94	147	,, ,,
	18	1·92	142	,, ,,
	27	1·92	151	,, ,,
	7	1·89	141	+
	10	1·88	142	+
	31	1·86	152	+
	37	1·83	156	+ (Tabby cat heart).
	13	1·82	124	+ +
	21	1·80	143	Brown atrophy.
	25	1·77	155	,, ,,
	12	1·66	144	,, ,,
	9	1·65	141	,, ,,
	33	1·61	159	,, ,,
	35	1·60	133	+
	19	1·57	140	+
	26	1·40	141	+
Average of 17 cases with less than 2 per cent. in heart.		**1·76**	**144**	

TABLE IV.—*Cases grouped according to Amount of Fat in Kidney (Adults).*

	Case No.	Per cent. of Fat in Kidney.	Iodine Value.	Histological Notes.
A . .	36	3·72	105	+ + -
	30	3·25	126	--
Average of 2 cases over 3 per cent.		**3·49**	**116**	
B . .	10	2·54	126	+ -- -
	23	2·41	119	--
	39	2·40	117	+ + +
	24	2·27	129	+ + +
	42	2·03	146	+
	6	2·01	129	+ + +
Average of 6 cases over 2 per cent.		**2·28**	**128**	
C . .	35	1·92	137	+
	31	1·92	123	+ +
	38	1·90	131	No sections.
	12	1·85	135	+
	2	1·84	111	+ +
	10	1·84	125	+
	19	1·84	123	+
	29	1·84	137	No sections.
	18	1·80	124	+ +
	8	1·79	130	+
	17	1·76	127	+
	7	1·70	137	+ +
	25	1·68	141	+ +
	13	1·67	130	+ (?)
	28	1·60	134	+
	33	1·60	143	0
	3	1·55	132	No sections.
	9	1·52	134	0
Average of 19 cases over 1·5 per cent.		**1·76**	**131**	
D . .	11	1·47	133	+
	1	1·44	139	No sections.
	27	1·43	157	+ +
	22	1·38	125	+ + +
	37	1·22	142	+
	16	1·32	138	+
	26	1·31	137	+
Average of 7 cases under 1·5 per cent.		**1·37**	**139**	

[HISTOLOGICAL PROTOCOLS.

HISTOLOGICAL PROTOCOLS.

Case 1.—No sections were examined.

Case 2 (Pernicious Anæmia).—*Heart*—A large amount of granular fat, patchy in its distribution throughout the heart muscle. In such areas the granules are present in all parts of the cell cytoplasm, 2·62 per cent.; iodine value, 112. *Kidney*—A moderate amount of fat present in the epithelium lining the tubules, most marked in the straight tubules, 1·84 per cent.; iodine value, 111. *Liver*—Moderate amount of fat; fine granular forms distributed throughout the cells of the lobules, with larger globules located about the periphery of the lobules, 4·14 per cent.; iodine value, 117.

Case 3 (Pernicious Anæmia).—*Heart*—Fat unevenly distributed, and where it is present the cells are markedly enlarged, 3·77 per cent.; iodine value, 109. *Kidney*—1·55 per cent.; iodine value, 132. *Liver*—5·69 per cent.; iodine value, 107.

Case 4 (Anæmia in a Child).—*Heart*—Shows a large amount of fat, fairly even in its distribution, 2·87 per cent.; iodine value, 97. *Kidney*—A small amount of fat quite irregular in its location, 1·73 per cent.; iodine value, 128. *Liver*—A large amount of fat present for the most part in the form of large globules, which in many areas extend throughout the whole lobule to the intra-lobular veins, 6·09 per cent.; iodine value, 85.

Case 5 (Infarct with Abscess in Thymus Gland).—*Heart*—Showed no fat whatever, 2·19 per cent.; iodine value, 108. *Kidney*—Small amount of fat in local areas, 2·41 per cent.; iodine value, 108. *Liver*—3·18 per cent.; iodine value, 95.

Case 6 (Pneumonia).—*Heart*—A moderate amount of fine granular fat, also marked brown atrophy, 2·43 per cent.; iodine value, 136. *Kidney*—2·01 per cent.; iodine value, 129. *Liver*—Shows a moderate amount of fat, 2·86 per cent.; iodine value, 159.

Case 7 (Cause of Death Unknown). — *Heart*—Very small amount of fine droplet fat, with much brown atrophy, 1·89 per cent.; iodine value, 141. *Kidney*—Moderate amount of fatty changes, somewhat patchy in distribution, 1·70 per cent.; iodine value, 137. *Liver*—A very large amount of fat, both finely granular and large globular forms, extending irregularly throughout the whole lobule in some areas; 11·19 per cent.; iodine value, 84.

Case 8 (Lobar Pneumonia).—*Heart*—No fat present, but great majority of the cells show some brown atrophy, 1·99 per cent.; iodine value, 131. *Kidney*—Very small amount of demonstrable fat, 1·79 per cent.; iodine value, 130. *Liver*—Abundance of fat, fine granules throughout the cells of the lobules, with large droplets in the cells at the periphery of the lobule, 8·18 per cent.; iodine value, 87.

Case 9 (Typhoid Fever).—*Heart*—Shows no fat, but small amount of brown atrophy, 1·65 per cent.; iodine value, 141. *Kidney*—Very small amount of fat, 1·52 per cent.; iodine value, 134. *Liver*—Small amount of finely granular fat, 2·46 per cent.; iodine value, 131.

Case 10 (Lobar Pneumonia).—*Heart*—Small amount of fine droplet fat in focal areas, and much brown atrophy, 1·88 per cent.; iodine value, 142.

Kidney—Moderate amount of fat present, particularly in the straight tubules, 1·84 per cent.; iodine value, 125. *Liver*—Small amount of fine granular fat centrally located in the lobule, 2·98 per cent.; iodine value, 137.

Case 11 (Typhoid Fever).—*Kidney*—Small amount of fat in focal areas, 1·47 per cent.; iodine value, 133. *Liver*—Small amount of fine granular fat, 2·99 per cent.; iodine value, 120.

Case 12 (Fractured Cranium).—*Heart*—No visible fat, but marked brown atrophy, 1·66 per cent.; iodine value, 144. *Kidney*—Small amount of fat, 1·85 per cent.; iodine value, 135. *Liver*—Quite a moderate amount of fat, 4·49 per cent.

Case 13 (Malignant Endocarditis).—*Heart*—Small amount of fine droplet fat, 1·82 per cent.; iodine value, 124. *Kidney*—Small amount of fine droplet fat, 1·67 per cent.; iodine value, 130. *Liver*—Moderate amount of fat centrally located in the lobules, 2·74 per cent.; iodine value, 127.

Case 14 (Fœtus, Six Months).—*Heart*—Moderate amount of fine granular fat evenly distributed throughout the cells, 1·32 per cent.; iodine value, 148. *Kidney*—No demonstrable fat, 1·36 per cent.; iodine value, 125. *Liver*—Moderate amount of fat present, 1·38 per cent; iodine value, 132.

Case 15 (Infant).—*Heart*—No demonstrable fat, 1·21 per cent.; iodine value, 162. *Kidney*—No demonstrable fat, 1·31 per cent.; iodine value, 142. *Liver*—Few granules of fat present, 1·90 per cent.; iodine value, 143.

Case 16 (Pneumonia).—*Heart*—Occasional fibres show large amount of fat, though, on the whole, not much fat is seen, 2·52 per cent.; iodine value, 131. *Kidney*—Very small amount of fat, 1·32 per cent.; iodine value, 138. *Liver*—2·75 per cent.; iodine value, 139.

Case 17 (Hydroquinone Poisoning).—*Heart*—Very large amount of fat, 3·3 per cent.; iodine value, 120. *Kidney*—Small amount of fat, 1·76 per cent.; iodine value, 127. *Liver*—Moderate amount of fat, centrally located in the lobules, 3·45 per cent.; iodine value, 126.

Case 18 (Typhoid Fever).—*Heart*—No evidence of fat, but a large amount of brown atrophy, 1·92 per cent.; iodine value, 142. *Kidney*—Moderate amount of fat present, 1·80 per cent.; iodine value, 124. *Liver*—Considerable amount of fat present in both fine granules and larger droplets, 4·65 per cent.; iodine value, 116.

Case 19 (Tuberculosis, Pulmonary).—*Heart*—Small amount of fine droplet fat, uneven in its distribution, 1·57 per cent.; iodine value, 140. *Kidney*—Very small areas of fine granular fat, 1·84 per cent.; iodine value, 123. *Liver*—Very small amount of fine granular fat, 2·54 per cent.; iodine value, 134.

Case 20 (Fœtus, Seven Months).—*Heart*—1·26 per cent.; iodine value, 132. *Kidney*—Very small amount of fat, 1·71 per cent.; iodine value, 138. *Liver*—Moderate amount of fat, patchy in its distribution, 2·58 per cent.; iodine value, 103.

Case 21 (Toxæmia of Pregnancy).—*Heart*—No visible fat, but considerable brown atrophy, 1·80 per cent.; iodine value, 143. *Kidney*—Small amount of fat present, 1·48 per cent. *Liver*—Moderate amount of small granular fat, 3·27 per cent.; iodine value, 115.

Case 22 (CARCINOMA OF THE PANCREAS).—*Heart*—Large amount of fat, droplets rather large, 3·11 per cent.; iodine value, 122. *Kidney*—1·38 per cent.; iodine value, 125. *Liver*—Moderate amount of fat in lobules and in the carcinomatous areas, 3·21 per cent.; iodine value, 131.

Case 23 (TUBERCULOSIS).—*Heart*—Large amount of fat, 2·8 per cent.; iodine value, 118. *Kidney*—Very small amount of fat, 2·41 per cent.; iodine value, 119. *Liver*—Very large amount of fat, 12·81 per cent.; iodine value, 78.

Case 24 (PANCREATITIS, CHRONIC).—*Heart*—Very few fat granules, but considerable brown atrophy, 2·09 per cent.; iodine value, 144. *Kidney*—Moderate amount of fatty change, 2·27 per cent.; iodine value, 129. *Liver*—Very large amount of fat, 10·64 per cent.; iodine value, 79.

Case 25 (TYPHOID WITH ORBITAL CELLULITIS AND MENINGITIS).—*Heart*—No demonstrable fat, but moderate degree of brown atrophy, 1·77 per cent.; iodine value, 155. *Kidney*—Very small amount of fat present, 1·68 per cent.; iodine value, 141. *Liver*—Large amount of fat, 6·37 per cent.; iodine value, 105.

Case 26 (PUERPERAL SEPTICÆMIA).—*Heart*—Small amount of fine droplet fat and some brown atrophy, 1·40 per cent.; iodine value, 141. *Kidney*—Small amount of fatty change, 1·31 per cent.; iodine value, 137. *Liver*—Small amount of fat demonstrable, 2·90 per cent.; iodine value, 133.

Case 27 (CHRONIC ALCOHOLIC, DEATH FOLLOWING GASTRO-ENTEROSTOMY). *Heart*—No visible fat, but large amount of brown atrophy, 1·92 per cent.; iodine value, 151. *Kidney*—Small amount of fat, 1·43 per cent.; iodine value, 157. *Liver*—Moderate amount of fat, for the most part in the cells about the periphery of the lobules, 2·95 per cent.; iodine value, 139.

Case 28 (TUBERCULOSIS).—*Heart*—Small amount of fat, patchy in its distribution, 2·2 per cent.; iodine value, 127. *Kidney*—Small amount of fat present, 1·6 per cent.; iodine value, 134.

Case 29 (PNEUMONIA).—*Heart*—No visible fat, 1·94 per cent.; iodine value, 147. *Kidney*—1·84 per cent.: iodine value, 137. *Liver*—Abundant fat present, 10·31 per cent.; iodine value, 81.

Case 30 (ADDISON'S DISEASE).—*Heart*—Very small amount of fat, 2·04 per cent.; iodine value, 121. *Kidney*—Moderate amount of fat, 3·25 per cent.; iodine value, 126. *Liver*—Very abundant fatty change, 11·75 per cent.; iodine value, 83.

Case 31 (LOBAR PNEUMONIA, MENINGITIS).—*Heart*—No visible fat, but considerable brown atrophy, 1·86 per cent.; iodine value, 152. *Kidney*—Moderate amount of fat, 1·92 per cent.; iodine value, 123. *Liver*—Abundant fat present, 8·31 per cent.; iodine value, 94.

Case 32 (MILIARY TUBERCULOSIS).—*Heart*—Moderate amount of fat present, 2·18 per cent.; iodine value, 118. *Liver*—Small amount of demonstrable fat, 3·02 per cent.; iodine value, 131.

Case 33 (PNEUMONIA).—*Heart*—No demonstrable fat, some brown atrophy, 1·61 per cent.; iodine value, 159. *Kidney*—No fat present, 1·60 per cent.; iodine value, 143. *Liver*—Small amount of fat present, 3·14 per cent.; iodine value, 140.

Case 34 (GASTRIC CARCINOMA).—*Heart*—No demonstrable fat, but considerable brown atrophy, 2·12 per cent.; iodine value, 148. *Kidney*—Moderate amount of fat, 1·93 per cent.; iodine value, 131. *Liver*—Moderate amount of fat, 4·75 per cent.; iodine value, 85.

Case 35 (LOBAR PNEUMONIA).—*Heart*—Marked brown atrophy, but no fat, 1·60 per cent.; iodine value, 133. *Kidney*—Small amount of fat, 1·92 per cent.; iodine value, 137. *Liver*—Very large amount of fat, 15·41 per cent.; iodine value, 76.

Case 36 (DIABETES WITH LIPÆMIA).—*Heart*—Shows a moderate amount of fat, some of which is intracellular, but the greater portion is present in the intercellular capillaries, 2·36 per cent.; iodine value, 132. *Kidney*—A large amount of fat present in the epithelium lining the tubules, and in the blood vessels and capillaries of the kidney, 3·72 per cent.; iodine value, 105. *Liver*—Shows quite a large amount of fat, there is some fine granular fat within the cells, but most of the fat is present in the intercellular spaces and so marking out the cellular structure of the organ, 5·93 per cent.; iodine value, 104.

Case 37 (SEPTICÆMIA).—*Heart*—Small amount of fat, but to naked eye typical "tabby-cat" markings, 1·83 per cent.; iodine value, 156. *Kidney*—Very small amount of fat, 1·22 per cent.; iodine value, 142. *Liver*—Small quantity of fat, 2·66 per cent.; iodine value, 134.

Case 38 (PERNICIOUS ANÆMIA).—No sections.

Case 39 (MALIGNANT ENDOCARDITIS).—*Heart*—Large amount of fine granular fat, 2·59 per cent.; iodine value, 133. *Kidney*—Large amount of fat, 2·40 per cent.; iodine value, 117. *Liver*—Abundant fatty changes, 6·66 per cent.; iodine value, 109.

Case 40 (CHRONIC ALCOHOLISM).—*Heart*—Large amount of fat, 2·97 per cent.; iodine value, 128. *Kidney*—Large amount of fat, 2·54 per cent.; iodine value, 126. *Liver*—Very abundant fatty infiltration, 21·9 per cent.; iodine value, 70·9.

Case 41 (CHOLECYSTENTEROSTOMY).—*Heart*—Very large amount of fat, 3·36 per cent.; iodine value, 119·3. *Liver*—Abundant infiltration of fat, 7·83 per cent.; iodine value, 85.

Case 42 (SEPTIC PERITONITIS).—*Heart*—Fine granular fat in all the cells; 2·19 per cent.; iodine value, 138. *Kidney*—Small amount of fat, 2·03 per cent.; iodine value, 146. *Liver*—Moderate amount of fat, 3·80 per cent.; iodine value, 118.

Reprinted from THE JOURNAL OF BIOLOGICAL CHEMISTRY, Vol. XX, No. 1, 1915

ON THE FAT IN THE BLOOD IN A CASE OF LIPAEMIA.

BY C. G. IMRIE.

(*From the Department of Pathological Chemistry of the University of Toronto.*)

(Received for publication, November 28, 1914.)

A patient was admitted into the Toronto General Hospital in March, 1914, who up to that day had been at work as a labourer, and eight hours after admission died in a state of coma.

At the autopsy it was observed that the blood that escaped in the first incision was remarkably pale in colour, appearing like cream on dividing vessels in the least dependent parts of the cadaver; in more dependent parts it was redder, but still evidently rich in fat. In the pancreas a nodular projection was found in front of the spinal column, and extensive induration, interlobular in distribution and of a more chronic character, stretched throughout the tail of the organ. A full account of the microscopical anatomy of this, as well as of a remarkable condition in the spleen of this patient, is given elsewhere by Prof. J. J. Mackenzie.[1] Blood was collected from the heart and great vessels of the thorax, and urine from the bladder.

In the urine sugar was found amounting to 6.2 per cent; and from 50 cc., acidified with sulphuric acid and saturated with ammonium sulphate, ether in a continuous extraction apparatus extracted a laevorotatory acid which in its rotatory power was equivalent to 0.67 per cent of β-hydroxybutyric acid in the urine. The ammonia coefficient was 11.9. In addition, ether extracted from the urine 0.1 per cent of acids insoluble in water, the molecular weight of which by titration was found to be 282, and which in other ways too appeared to be higher fatty acids. With them was obtained also a trace of cholesterol amounting to about 3 mgm. from 50 cc. of urine.

The blood was put in a separating funnel and the creamy serum separated from the corpuscles. Its specific gravity at 16.5°C.

---

[1] J. J. Mackenzie: unpublished report.

was 1014. Of this serum 109.5 grams were treated with 5 cc. of 40 per cent potash and about 100 cc. of petroleum ether and as much alcohol. After being shaken, the clear petroleum solution was removed and the shaking was repeated three times with fresh portions of petroleum ether. A fifth portion of petroleum ether used in the same way was found to have removed only 2.5 mgm. of soluble material. The mixed petroleum solutions were made up to 250 cc. In a portion of this the dissolved matter was determined to be 14.06 per cent of the serum by weight. This residue was saponified with alcoholic potash and the alkaline solution shaken with petroleum ether several times. Unsaponifiable substances thus removed amounted to 1.5 per cent of the serum by weight.

The alkaline serum was then made acid and again shaken with petroleum ether, which now removed a further quantity of soluble matter, fatty acids, originally present in the serum as soaps, and amounting to 0.38 per cent of the serum.

Thus there were found in the serum:

A. Neutral fat and cholesterol, 14.06 per cent.
B. Cholesterol (unsaponifiable matter), 1.5 per cent.
C. Fatty acids originally in the form of soaps, 0.38 per cent.

Fraction A. Neutral fat and cholesterol gave the iodine value, by Wijs's method, 78.6, and the saponification value, after deducting cholesterol, 190; the amount of insoluble fatty acids obtained by saponification of this portion was 91.2 per cent of the neutral fat, and these acids then had the iodine value 73.0. The products of saponification soluble in acid obtained from 2 grams of the extract were boiled with nitric acid for some time and examined for phosphoric acid, but no phosphomolybdate of ammonia was formed.

Fraction B, containing the cholesterol, was remarkably colourless and crystallized as if it were almost pure cholesterol. A solution of it in alcohol containing 0.2237 of a gram was precipitated with digitonin by Windaus's method, and yielded a precipitate of the cholesteride corresponding to 0.2190 of a gram of free cholesterol; so esters of cholesterol cannot have been present in appreciable amounts. The iodine value of the unsaponifiable matter when first obtained was 118 by Wijs's method; seven months later when determined again by this method it had fallen to 89, but by Hübl's method the theoretical value for pure cholesterol was then given; *viz.*, 65. The melting point of the unsaponifiable matter without being purified by recrystallization was 141–142°C. Pure cholesterol melts at 145–146°.

Fraction C, the fatty acids originally present in the serum as soaps, gave the iodine value 88.6.

Some of the subjacent corpuscular layer was heated with potash and alcohol, as in Liebermann's method of fat estimation; on acidification and shaking with petroleum, fatty acids were obtained amounting to 0.15 per cent, which is not more than is found in normal blood corpuscles.

1. The fat in the blood of this case was present, therefore, in large amount: larger amounts have been recorded; for instance, 18 per cent (Fischer),[2] 19.7 per cent (Neisser and Derlin),[3] 29 per cent (Adler).[4] But it was evident that the amount could vary considerably according to the level in the body of the vessels from which the blood was obtained.

2. The fat was composed almost entirely of simple glycerides and contained but little if any of the phospholipines, such as lecithin. For on saponification the fat yielded 91.2 per cent of insoluble higher fatty acids, whereas lecithin with the conventional formula yields at most 70 per cent, other phospholipines less still, and the simple glycerides of adipose tissue about 95 per cent. The fact that less than this amount was obtained may be explained by supposing that a small amount of some lower soluble acids, such as butyric acid, took part in the composition of the glycerides, and need not indicate the presence of phospholipines, the absence of which was made clear by the failure to detect any phosphoric acid.

3. The fatty acids entering into the composition of the fat, as shown by their iodine value 73, were similar to those obtained from adipose tissue (iodine value about 65) and quite different from those normally found in the liver, heart, or other organs. The liver of this same patient gave 5.7 per cent of fatty acids, by the same treatment and method of estimation, having the iodine value 104 (this fat in the liver was much more conspicuous in the capillaries than in the cells), the heart gave 2.36 per cent, with iodine value 132, and the kidney 3.72 per cent, a large amount for this organ, with iodine value 105. These data point to the cause

---

[2] B. Fischer: *Virchows Arch. f. path. Anat.*, clxxii, pp. 30 and 218, 1903.

[3] E. Neisser and L. Derlin: *Ztschr. f. klin. Med.*, li, p. 428, 1904.

[4] M. Adler: *Berl. klin. Wchnschr.*, xlvi, p. 1453, 1909.

of the lipaemia in this case being the mobilization of connective tissue fat; the other possibility, accumulation in the blood of fat absorbed from the food, is rendered improbable by the large amount, which in the whole blood can hardly have been less than 300 grams and was probably more.

4. The amount of soap in the blood is striking; though in comparison with the amount of neutral fat it is small, absolutely as much fatty acid was present in this form as is often present in all kinds of combination in normal blood.

5. The amount of cholesterol, 1.5 per cent of the serum, rather more than one part for every ten parts of fat in the blood, is also striking; but still larger amounts have been found; *e.g.*, 2.6 and 3.6 per cent (Adler).

6. No evidence of the presence of lecithin in the serum was obtained. The tendency for the amount of lecithin in the blood in diabetic lipaemia to be small has been pointed out by Adler and by Klemperer and Umber.[5]

The above work was carried out under the direction of Prof. J. B. Leathes, to whom I am much indebted for his kind interest and suggestions.

---

[5] G. Klemperer and H. Umber: *Ztschr. f. klin. Med.*, lxv, p. 340, 1908.

UNIVERSITY OF TORONTO STUDIES

MEDICAL RESEARCH FUND

No. 1:	On the haemolytic properties of fatty acids and their relation to the causation of toxic haemolysis and pernicious anaemia, by W. F. McPhedran	1.00
No. 2:	Biological curves obtained during the onset and course of tuberculous infection, by A. H. Caulfeild and F. S. Minns	
No. 3:	Tuberculin treatment based upon clinical and biological data, by A. H. Caulfeild and F. S. Minns	
No. 4:	The excretion of nitrogen in fever, by N. C. Sharpe and K. M. B. Simon	
No. 5:	On fatty changes in the liver, heart and kidney, by C. G. Imrie	
No. 6:	On the fat in the blood in a case of lipaemia, by C. G. Imrie	